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ORAL HABITUATION AND THE CONTROL OF
INGESTIVE BEHAVIORS IN RATS

by

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Dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in the Department of Experimental Psychology
in the Graduate School of Duke University

ABSTRACT

(Psychology - Biological)

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ABSTRACT

Previous studies of ingestion in rats documented changes in ingestive behavior attributed to consequences of associative learning, but little attention has been focused on the influences of ongoing experience within an ingestive episode. In the studies reported here, oral experience was provided to rat pups in a series of brief intra-oral infusions and steady decreases in the level of oral responsiveness were observed. This habituation of mouthing responses resulted in long-lasting suppression of actual intake that was specific to the diet experienced orally. When intervals between diet stimulus infusions were relatively long (circa 1 min), suppression was evident even 3 hrs after habituation experience. A short-lasting decremental process modulated oral activity when inter-stimulus intervals were short, but the effect was brief (lasting less than 30 minutes). During the course of this oral habituation, patterns of electromyographic activity in masticatory and tongue muscles, changed dramatically within and across trials and after oral habituation had developed, activity in 2 of the muscles was greatly reduced or abolished. The effects of ongoing oral experience were also found to be modulated by other ingestion-related physiological signals. Oral responsiveness in 6-hr deprived pups was modulated by gastric fill; gastric fill enhanced the expression of oral habituation. In contrast, gastric fill failed to affect habituation of mouthing responses in 24-hr deprived pups. Examination of the neural substrates of oral habituation in decerebrate pups revealed that the expression of oral habituation can be accomplished by the hindbrain. Further, decerebrate pups showed the capacity to integrate gastric fill and oral signals, but unlike neurologically intact pups, decerebrate pups demonstrated a response to gastric loads even after 24 hrs deprivation. Thus, while oral habituation and gastric fill modulation of habituation are subserved by the brainstem, the modulatory effects of deprivation on the integration of oral experience with gastric fill arise from the forebrain. The low level of neural representation of oral habituation

and its potent, long-lasting and diet-specific effects on consumption suggest that oral habituation may constitute a fundamental control that terminates ingestive episodes and that integrates other ingestion-related feedback signals.

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BACKGROUND

In many regards, and despite many decades of study, the control of feeding remains a mystery. Our inability to understand the controls of such a fundamental behavior may result foremost from the myriad influences on ingestive behavior in adult animals. For instance, the influences thought to modulate ingestion of adult rats range from physiological signals (i.e. gastric fill level, blood glucose level, etc.) to the experiential (i.e. conditioned taste aversions, prior learned associations between food and its post-ingestive consequences). That ingestion may be simpler and more easily understood at early stages in development than when physiological and neural maturation have added complexities is one of the appeals of a developmental study of ingestive behavior. In fact, recent data confirm that early controls of ingestion are more rudimentary (see Hall, 1990 for review). In this dissertation, I use the strategy of developmental analysis to assess the potential importance of ongoing experience to the control of ingestion.

What previous studies of controls of ingestion have shown.

The oral consummatory component of ingestion has been emphasized in developmental investigations to date. Analysis has focused on how internal physiological signals control behavior and began with the question: What does a newborn rat pup do with food or fluid placed in its mouth? Wirth and Epstein (1976) addressed this question by holding rat neonates to a flowing water spout. In such tests pups were not required to produce all the preceding components of the appetitive response sequence in order to indicate whether they possessed the substrates for adult-like lapping and swallowing. The procedure for studying this final component of ingestion was extended using controlled deliveries of test fluids through oral cannulas (Hall, 1979).

Controls are present in young pups

We have learned that from birth and earlier rat pups will actively lap, mouth, and swallow fluids (Hall, 1979; Johanson & Hall, 1980; 18-day fetuses, Smotherman & Robinson, 1987). This early independent ingestion is deprivation dependent, with pups as young as 1 day of age showing increased intake with increased deprivation from the mother and milk. Not only do neonates ingest orally infused diets, they will also consume solutions spread on the floor of their warm test containers (Hall & Bryan, 1980). The termination of ingestion in both test situations has behavioral parallels to the satiety sequence seen in adult animals (Antin, Gibbs, Holt, Young & Smith, 1975). In addition, ingestion in response to oral infusions is influenced in an adult-like fashion by sensory properties of food (e.g. Hall, 1979; Ganchrow, Steiner & Canetto, 1986) even though considerable postnatal maturation of sensory systems occurs (e.g. olfactory physiology, Brunjes & Frazier, 1986; taste physiology, Hill, Bradley & Mistretta, 1983; Hill, 1987; taste preference, Hall & Bryan, 1981; Vigorito & Sclafani, 1988; Bernstein & Courtney, 1987; Moe, 1986). In short, young pups appear to possess neural substrates and effector systems needed for the oral response component of adult-like ingestion, despite the fact that they normally do not eat solid food until the third postnatal week.

But, controls in young pups are rudimentary

It now appears that in young pups, the primary stimulatory signal for ingestion may be dehydration while gastric fill may be the only postingestive inhibitory signal for ingestion. Acute, experimentally-induced dehydration has long been viewed as a potent stimulus for independent ingestion in neonatal rats (Wirth & Epstein, 1976; Bruno, 1981; Bruno, Blass & Amin, 1983). But dehydration after milk deprivation was not viewed as an essential stimulus for ingestion because in previous studies, deprivation-induced dehydration (cellular and extracellular; Bruno, 1981; Friedman, 1979) was confounded with nutritional deficits and

empty stomachs. When the confound of dehydration was removed in 6-day-old, food-deprived pups, no increase in ingestion was found after deprivation (Phiher, Ladd & Hall, 1991) showing that the essential stimulus in a 6-day old was dehydration, not a nutrient deficit or an empty stomach. However, when the same procedure was used in 15-day-old pups, hydrated pups consumed just as much as deprived control pups (Phiher, Ladd & Hall, 1991). By this age, there appears to be a stimulus for ingestion after deprivation other than dehydration.

While dehydration stimulates ingestion in young pups, the primary postingestive inhibitory control of intake appears to be gastric fill. Deprived 6-day-old rats with closed pyloric nooses that prevent movement of ingested solutions from the stomach to the intestines ingested volumes similar to those of pups ingesting normally. Therefore, stomach fill alone was sufficient to stop ingestion (Phiher & Hall, 1988). In addition, and in contrast to adults (e.g. Deutsch, 1985), inhibition resulting from gastric fill was a product of the amount rather than the chemical makeup of the solution that fills the stomach. Milk, isotonic saline, and glucose preloads all resulted in intake termination at an equivalent point of gastric fill in young pups with occluded pyloruses.

Complexities are added as pups mature

Providing pups with caloric gastric loads revealed new influences on the ingestive responses of 15-day olds. Preloads of milk and glucose reduced intake relative to saline loads. More importantly, and similar to adults (Kraly & Smith, 1978), these loads resulted in a termination of ingestion at lower volumes of gastric fill (Phiher & Hall, 1988). This finding indicates that by 15 days of age there is an inhibitory modulatory signal that is postgastric. And I have shown that while this postgastric modulatory signal is apparent as early as 9 days of age, its effects become more pronounced in older pups (e.g. 12- and 15-day olds;

Swithers & Hall, 1989a). Further, the postgastric effects of nutritive loads are specific to diets which the pups readily metabolize. For example, sucrose loads are not readily absorbed by pups tested at 9 days of age because the sucrose digesting enzyme sucrase is not active at this age (e.g. Henning, 1987). And, in 9-day-old pups, preloads of sucrose have no effect on intake, but the nutritive constituents of sucrose, glucose and fructose, do inhibit ingestion (Swithers and Hall, 1989b). By 21 days of age when appreciable amounts of sucrase activity are present, sucrose preloads inhibit ingestion. These results suggest that the postgastric effects of a nutritive load in young animals are unlikely to be due to illness resulting from the preload procedure and instead appear to result from post-absorptive signals.

What's missing from the developmental analysis of ingestive controls

The results I've summarized describe an analysis of how internal physiological signals influence independent ingestive behavior in developing rat pups. What these studies have neglected, however, is the influence of experience on control of ingestion. In particular, the effects of the ongoing oral stimulation that characterizes ingestion have not been explored. Examples of controls of behavior that depend on previous activation of that behavior are ubiquitous in other response systems, (e.g. habituation of sexual responses of male rats following copulation, Fowler & Whalen, 1961; habituation of mobbing response of chaffinches, Hinde, 1970). From an ethological perspective, it is reasonable to suppose that the performance of a behavior such as ingestion might have an impact on the subsequent ingestive behavior (e.g. Thorpe, 1951; Tinbergen, 1950). Thus far, such experiential controls have not been thoroughly explored for their potential contributions to the control of ingestion. The set of studies described below specifically examines how ongoing performance of the oral consummatory component of ingestion affects subsequent

performance of ingestive behaviors.

THE EXPERIMENTS

The opening chapter of this dissertation details my experiments describing the basic phenomena in 6-, 12- and 18-day-old rat pups; when oral experience is emphasized, and post-ingestive signals are minimized the mouthing responses of pups to a series of brief, intra-oral infusions decline rapidly. Similar patterns of response decrement have been described in a number of responses systems, and in other systems are termed habituation. The results presented in Chapter 1 indicate that in rat pups, the mouth habituates and that the pattern of oral habituation is dependent on both the pup's state (deprivation level) and its age.

In Chapter 2, the effects of oral habituation on actual consumption of diet are tested in two different types of tests designed to examine the entire sequence of feeding or the oral consummatory component alone. Although the decrease in duration of mouthing activity demonstrated in Chapter 1 presumably reflected a general decrease in ingestive responsiveness, but in Chapter 1 no measures of actual intake were obtained. In Chapter 2, oral habituation is seen to diet-specifically suppress intake in two separate ingestive tests. In addition, diet-specific intake-suppressing effects are apparent 30 minutes following oral habituation experience and intake is non-specifically suppressed even 3 hrs after oral habituation experience. The duration of this effect depends on the rate of stimulus presentation during oral habituation; mouthing responses continue to decline with shorter interstimulus intervals, but the effects last less than 30 minutes.

Chapter 3 describes an electromyographic analysis of the oral habituation in four masticatory and tongue muscles: the superficial masseter, anterior digastric, sternohyoideus and genioglossus. These results demonstrate that the pattern of muscle activity changes both within trials and during the course of habituation. In particular, the cycle frequency is

reduced at the end of a trial compared to the beginning of that trial, and is reduced after the development of habituation compared to at the beginning of testing. Within a trial, the duration of muscle activity within a cycle is shorter at the start of the trial compared to the end. By the end of habituation, muscle activity durations are considerably lengthened and phase relationships between the muscles are dramatically altered. Especially striking is the abolishment or substantial reduction of activity in the genioglossus and sternohyoideus while the masseter and digastric continue to be active.

The neural substrates for oral habituation are examined in Chapter 4. The possibility that the brainstem is sufficient for the production of oral habituation is tested in decerebrate pups and indeed, decerebrate pups continue to demonstrate oral habituation. However, the overall lower responding of decerebrates compared to control pups and the lack of sensitization in decerebrates suggest some forebrain influences on responsiveness in intact rats.

Chapter 5 describes the integration of physiological signals related to gastric fill and deprivation and the neural bases for this integration. Oral responding is demonstrated to decline more rapidly in mildly (6-hr) deprived pups that receive both oral and gastric infusions compared to pups that receive only oral infusions. More rapid decreases in oral responsiveness are also seen in decerebrate pups even following more lengthy deprivation periods. Mouthing responses of decerebrate pups deprived for 24 hrs prior to habituation experience and receiving both oral and gastric infusions decrease more quickly than in 24-hr deprived decerebrate pups receiving oral infusions alone. However, neurologically intact pups tested after 24 hrs deprivation fail to demonstrate more rapid decreases in oral activity when receiving both oral and gastric infusions compared to oral infusions alone. These results suggest that the brainstem alone integrates oral experience and gastric fill signals, but that the modulatory influence of deprivation which prevents gastric fill from enhancing

response decrements in intact pups originates in the forebrain.

The final chapter of presents a theoretical review of present theories of oral controls of ingestive behavior and incorporates the major findings from my studies in developing rat pups into an alternative perspective which ascribes a major, integrative role to oral processes.

CHAPTER I.
HABITUATION OF OROMOTOR RESPONDING TO ORAL
INFUSIONS IN RAT PUPS

ABSTRACT

The influence of oromotor experience on the pattern of ingestion in rat pups and the relation of this influence to age and pups' physiological state were investigated using a procedure designed to mimic the sham-feeding preparation in adult rats. Six-, 12-, and 18-day-old pups received brief intra-oral infusions of sucrose solutions once every minute. Small infusion volumes minimized post-oral effects. Pups' oromotor responsiveness was assessed by recording the pattern of mouthing behavior continuously during the test. Pups were tested after 24, 6 or 0 hrs deprivation. During testing, the mouthing behavior of all pups except 24-hr deprived 6-day-olds showed a marked decline. The specificity of this decrement was demonstrated in a second experiment in which the decremented response was restored by a switch in solution flavor. Finally, the influence of post-oral signals on the decline in responsiveness was evaluated by comparing the oral responsiveness of 18-day-old pups following intra-gastric, oral, or no infusions. Oral infusions suppressed subsequent oral responding, but intragastric infusions did not. These results provide evidence for a habituation-like role of oromotor experience in determining patterns of ingestive behavior within a feeding test. Here, major determinants of the pattern of decline were pups's physiological state and developmental age.

INTRODUCTION

One useful strategy in unraveling the controls of ingestion is the study of their ontogeny. In developing animals, the controls of ingestion may be fewer and less complex, and thus more easily identified. In fact, it has been found that physiological controls of intake of orally infused solutions in very young rat pups are simple - intake is determined only by level of gastric fill and hydrational status (see review, Hall, 1990). As pups mature, more complex controls are added, including responsiveness to nutritive signals.

Little attention, however, has been directed toward understanding the role of ingestive experience in controlling early ingestive behavior. In addition to feedback signals from post-ingestive consequences, some control of ingestive behavior may be consequent upon ongoing ingestive experience (e.g. Hinde, 1970). In particular, the performance of oromotor responses during feeding may affect a pup's or adult's willingness to continue performing oromotor responses.

An approach to testing the influence of oromotor experience on subsequent willingness to ingest is to eliminate post-ingestive influences on the ingestive pattern. This is readily accomplished in adult rats by creating a fistula in the esophagus (Mook, 1963) or stomach (Young et al., 1974) so that ingested diet spills out without accumulating in the stomach or being absorbed, a procedure known as sham feeding. Sham-feeding preparations also have been utilized in 6-day-old pups. Behavioral observations in these animals indicated that when no post-ingestive effects of feeding are occurring, mouthing and licking persist throughout a one hr test (Phifer et al., 1986). These observations would appear to argue against an effect of oral experience in inhibiting or controlling early ingestion. However, because the sham feeding surgery necessitates at least an overnight recovery period during which the pups cannot be with their mother, sham feeding in pups can only be studied following a significant period of deprivation. The sustained responding

in these animals after such a procedure may be a result of the deprivation. To circumvent this difficulty we have developed a procedure that approximates sham feeding for rat pups by minimizing post-ingestive signals. In our method, the duration of mouthing behavior after a brief oral stimulus is used as a probe of a pup's appetitive state. This procedure has allowed us to explore the contributions of oromotor experience to the patterns of ingestion in pups under a range of deprivation conditions and across the preweaning period.

The present study examined the effects of oromotor experience within a feeding bout on the pattern of ingestion during that bout, and how this influence changes as pups mature and other controls of ingestion develop. Pups at 6, 12, or 18 days of age received a series of brief intra-oral infusions of sucrose. Potential post-absorptive signals were minimized in two ways. First, the small size of the diet infusions minimized gastric fill. Second, the diet chosen was a sucrose solution; pups at these ages have very low levels of intestinal sucrase activity and therefore do not readily metabolize sucrose (see Henning, 1987 for review).

We found that within a feeding test, even when post-ingestive signals were minimized, oromotor experience still produced a decline in ingestive behavior. While the effects of deprivation on the decrement in mouthing responses changed across the preweaning period, the decrement was stimulus-specific at all ages. Experience therefore exerts a control over ingestion from an early age and we conceptualize this effect of oral experience in terms of habituation.

GENERAL METHOD

Subjects

Subjects were offspring of primiparous and multiparous female Charles River CD

strain rats maintained in a breeding colony in our laboratory. After they were bred, pregnant females were housed individually in polyethylene cages during the week prior to parturition and remained with their litters until the time of testing. Cages were checked for births daily between 0800 and 1700 hrs and pups found before 1700 hrs were considered 0 days of age. Litters were culled to 10 pups (5 male and 5 female) at 1 day of age. Water and Purina Formulab Chow (no. 5008) were available ad lib in the cages. The colony room was maintained at 21-23° C (40-70% relative humidity) on a 14:10 light-dark cycle. Testing was conducted 2 hrs before the beginning of the dark cycle.

Deprivation

Pups in these experiments were tested after 24, 6 or 0 hrs deprivation. On the day before testing, pups to be tested at 24 hrs deprivation were removed from the mother's cage, placed in a polyethylene cage lined with absorbent bedding and housed inside a warm (32° C), humid incubator. On the day of testing, pups to be tested at 6 hrs deprivation were removed from their mother's cage.

Cannula Placement

Six hrs before testing, all experimental pups were removed from the dam or from the incubator and anesthetized with CO₂. Anterior oral cannulas were implanted under the tongue as previously described (Hall, 1979). Briefly, a 10 cm length of polyethylene tubing (PE-10), flanged on one end, was friction fitted to one end of a curved piece of stainless steel wire. The point of the curved end of the wire was placed into the pup's mouth and through the soft floor of the oral cavity just posterior to the lower incisors. The tubing was then drawn through the lower jaw until the flared end was seated against the inner surface of the mouth. This positioning permitted the pup to freely ingest or reject infused diets. After placement of the cannula, deprived pups were returned to the incubator; pups to be tested non-deprived were returned to their dam.

Ingestive tests

Immediately prior to testing, pups were stimulated to urinate and defecate by stroking their perineal region with a soft brush. Pups were then placed into clear plastic test containers (6.5 x 11 x 12.5 cm) inside a warm, humid glass incubator. The oral cannula was attached to a length of plastic tubing which connected to a 5 cc syringe mounted in a Harvard infusion pump. A programmable timer (Chroncontrol) controlled the action of the infusion pump. After a 15 minute accommodation period in the testing containers with the cannula leads attached, ingestive tests were begun. During testing, brief infusions of solution were delivered through the cannulas and pups' mouthing behavior was scored continuously. Mirrors behind and under the clear floor of the pups' test containers allowed for easy viewing of pups' oral activity. 'Mouthing' behavior was defined as opening or closing of the jaws and/or movement of the tongue and was recorded second by second.

EXPERIMENT 1: EFFECTS OF DEPRIVATION ON PATTERNS OF MOUTHING

Earlier sham feeding studies in pups suggested that, in the absence of post-ingestive effects, the oral responsiveness of 6-day-old pups remains high for some time if the test is conducted after 24 hr deprivation. Therefore, we examined the influence of the length of prior deprivation on mouthing in the absence of post-ingestive feedback. In addition, the role of prior deprivation in control of ingestive responding was studied in pups across the preweaning period.

Procedures

Pups were tested at 6, 12, or 18 days of age. In each litter, two pups (one male, one female) were tested after 24 hrs deprivation, two pups were tested after 6 hrs deprivation and two were tested non-deprived. Six litters of pups were used for testing at each age, (thus, n=12 in each condition). Each pup was tested only once.

During testing, pups received a brief infusion of a 10% (w/v) sucrose solution once a minute. Six-day-olds received 21 infusions of one second each (volume of each infusion approximated 0.0125 ml); 12- and 18-day-olds received 30 infusions of 3 seconds each (infusion volumes of 0.0200 ml and 0.0280 ml respectively). The total volume of solution infused by the end of testing was approximately equal to 2% of the pup's body weight. The pattern of pup's mouthing movements was recorded on a portable computer every second beginning with the start of the first infusion. When possible, observers were blind as to deprivation condition. For statistical analysis, first the total number of seconds mouthing during each one minute trial was computed for each pup and then the trials were averaged into 3 minute blocks. A one-way, repeated measures ANOVA was performed on the 3 trial block data at each age (Trials X Deprivation; PC-SAS, release 6.03, Statistical Analysis Systems, Cary, NC), and Tukey's Honestly Significant Difference (HSD) test was used for post-hoc comparisons between individual deprivation levels within each age, with a $p < 0.05$ taken as significant.

Results and Discussion

Length of prior deprivation had distinct effects on the oral responsiveness of 6-day-old pups (Fig. 1A; Deprivation, $F [2,31] = 38.96$, $p < 0.0001$). Twenty-four hr deprived pups responded vigorously to the first infusions and showed high levels of responding throughout testing. This pattern of responding is similar to the mouthing responses seen previously in pups sham feeding after 24 hrs deprivation (Phifer et al., 1986). In contrast to the sustained level of mouthing in 24-hr deprived pups (Trials X Deprivation, $F [12, 186] = 4.56$, $p < 0.0001$), the responsiveness of both 6-hr deprived and non-deprived 6-day-olds decreased during successive presentations of the diet (Trials, $F [6, 186] = 19.41$, $p < 0.0001$).

The mouthing patterns of 12-day-old pups were influenced by deprivation as well.

Initial mouthing behavior was more robust in 6 and 24 hr deprived 12-day-olds compared to non-deprived 12-day-olds, but there was little difference in responding between the two deprived groups (Fig. 1B; Deprivation, $F [2, 32] = 5.32, p < 0.01$). All 12-day-old pups showed a similar decrement in responsiveness over the course of testing (Trials X Deprivation, $F [18, 288] < 1, NS$).

For 18-day-old pups, deprivation appeared to have no effect on either the initial responsiveness to the infusions or on the slope of the decremented response (Figure 1C, Deprivation, $F [2, 31] < 1, ns$; Trials X Deprivation, $F [18, 261] < 1, ns$). All 18-day-old groups showed significantly lower response levels at the end of testing than at the beginning (Trials, $F [9, 261] = 23.81, p < 0.0001$).

These results show that during repeated stimulation pup's oromotor responsiveness to an infused diet decreases for at least some deprivation conditions and at all ages. Similar decreases in responsiveness to ingestive stimuli have been previously described in a number of other species. This type of intra-meal decrement has been termed sensory- or stimulus-specific or oropharyngeal satiety (Clifton, Burton & Sharp, 1987; Mook et al., 1980; Rolls et al., 1981a,b; 1982). Others, studying ingestion from an ethological perspective (e.g. in blowflies and chaffinches; Dethier, 1976; Prechtl, 1953) have noted the similarity of these stimulation-induced decrements in ingestion to the general process of habituation.

Habituation has in fact been described in a number of systems in rat pups and in adult animals in general. One possible explanation for the decrease in responsiveness in the present study is a habituation-like process. By this explanation, the rate of habituation is determined by both developmental age and the length of prior deprivation. While 24 hrs deprivation prevents a 6-day-old from rapidly habituating, by 18 days of age, prior deprivation has little effect on the habituation pattern.

While 'habituation' represents the most generalized mechanism to account for the

experiential effects producing the observed decline in mouthing behavior in these tests, other non-learning processes must be ruled out. Although the testing procedure was designed to minimize the post-ingestive influence of the diet on pups' behavior, the pups did consume the infused diet. It is possible that a gastrointestinal signal resulting from the consumption of the diet influenced the pups behavior. Another possibility is that by the end of testing, pups merely became fatigued. Experiment 2 was designed to further assess the importance of oral experience in the pattern of ingestive behavior.

EXPERIMENT 2: CONTRIBUTIONS OF ORAL FACTORS

The effects of altering the orosensory properties (specifically taste and smell) of the diet was examined in 6-, 12-, and 18-day-old pups. If orosensory or oromotor experience produces a habituation-like process that is responsible for the decline in pups' mouthing behavior seen in Experiment 1, then the decline in responsiveness should be specific to the diet being infused. Changing the orosensory properties of the infused diet after habituation has occurred should restore the response vigor. If, on the other hand, gastric fill or other direct post-ingestive signals, or fatigue, causes the decline in responsiveness, then ingestion of all diets should be depressed.

Procedures

Six hrs before testing, all pups were removed from the mother and had two anterior intra-oral cannulas implanted under CO₂ anesthesia. Pups were then placed in cages inside warm, humid incubators and deprived. At the time of testing, one of the pup's two cannulas was attached to a syringe filled with a grape flavored sucrose solution. The second cannula was attached to a syringe containing a cherry flavored sucrose solution. Both solutions were 5% sucrose (w/v) with 0.05% (w/v) unsweetened Kool-Aid added. The concentration of the sucrose solution was lowered from 10% in Experiment 1 to 5% in this experiment in

order to decrease the influence of taste of the sucrose and increase the possibility that the pups would be able to discriminate between the two flavors. During the first half of testing pups received brief infusions of one flavor of sucrose solution. Six-day-olds received 15 one-second infusions; 12- and 18-day-olds received 21 three-second infusions. During the second half of testing, 6-day-olds received 15 additional infusions and 12- and 18-day-olds received 21 additional infusions. The flavor of the infusions during the second half of testing was either the same as during the first set of infusions or was switched to the second flavor. The order of flavor presentation was counterbalanced across pups. At each age, pups from 6 litters were tested; each pup was tested only once. A one-way, repeated measures ANOVA (Trials X Switch) was performed on 3 minute blocks of data.

Results and Discussion

Because there were no differences between initial responding to the two flavors, the data for both flavors have been combined ($n=24$ for each group; 2 male, 2 female from each litter in each condition). During the first half of testing, pups at all ages showed a decline in mouthing responsiveness similar to that seen in response to unflavored sucrose solutions in 6-hr deprived pups (Figure 2A; 6-day-olds, Trials, $F [9, 405] = 109.62$, $p < 0.0001$; Figure 2B; 12-day-olds, $F [13, 598] = 107.85$, $p < 0.0001$; Figure 2C; 18-day-olds, $F [13, 598] = 26.43$, $p < 0.0001$). When the solutions were switched, pups at all ages exhibited a restoration of responding (6-day-olds, Trials X Switch $F [9, 405] = 6.3$, $p < 0.0001$; 12-day-olds, $F [13, 598] = 3.31$, $p < 0.001$; 18-day-olds, $F [13, 598] = 4.04$, $p < 0.001$). In 6- and 12-day olds, the restored response was significantly lower than the initial response level (6-day-olds, $p < 0.01$; 12-day-olds, $p < 0.01$), but levels of mouthing in 18-day-olds after a switch in diet were equal to those exhibited initially. In contrast, the responses of pups receiving the same flavored solution remained low.

The restoration of mouthing responsiveness following a change in the sensory properties of the diet indicates that the decrement in responding is diet-specific. This specificity demonstrates the importance of oral experience and suggests that post-ingestive consequences are an unlikely explanation for the decrement. The absence of complete restoration of responding in 6- and 12-day-olds may be attributable to generalization between the two diets, to fatigue, or to a gastrointestinal signal that may have exerted some effect. Experiment 3 was designed to assess the role of gastric and post-gastric signals on the pattern of mouthing.

EXPERIMENT 3: CONTRIBUTIONS OF GASTROINTESTINAL SIGNALS

Although in Experiment 1 post-oral accumulation and absorption were minimized, it is possible that some signal from the gastrointestinal system was responsible for the decrement in mouthing behavior observed in all but the 24-hr deprived 6-day-olds. To test this possibility, the responsiveness of 18-day-old pups given yoked intra-gastric infusions of sucrose was compared to the responsiveness of pups who received the sucrose infusions orally.

Procedures

Six hrs prior to testing, 18-day-old pups were removed from the mother and an indwelling gastric cannula was implanted under methoxyflurane (Metofane, Pitman-Moore) anesthesia using a modification of previously described procedures (Phiher & Hall, 1987). Briefly, a guide tube of 27 gauge syringe tubing with a soft rubber tip (Silastic tubing) was passed down the esophagus and into the stomach. One end of a length of stainless steel wire was inserted through the guide tube and out through the wall of the stomach and left abdominal wall. The guide tube was then withdrawn from the pup's mouth and to the end of the wire extending from the pup's mouth was friction fitted a length of PE-10 tubing. The

other end of the PE-10 tubing was flanged by heating. The wire and attached tubing were then gently pulled down through the stomach and abdominal wall until the flange rested against the inside surface of the stomach. An oral cannula was also implanted at this time as described above. Pups were then housed individually in plastic cages inside a warm incubator until the time of testing.

Before testing, 6 pups from each litter were placed into individual test containers and their oral and intragastric cannulas were connected to syringes containing the sucrose solution. They were allowed to accommodate to the containers for 15 minutes. During the first part of testing, two pups (Group Oral/Oral) received 30 oral infusions of a 10% sucrose solution, once a minute, as described in Experiment 1. At the same time, two pups received identical infusions through the intragastric cannula (Group Gastric/Oral). Two pups received no infusions during the first part of testing (Group No Infusion/Oral). Following the 30 infusions, the intragastric infusions were terminated, and all pups received 30 intra-oral infusions. Mouthing behavior was recorded continuously during the entire test. Immediately following testing, pups were sacrificed with CO₂ and intragastric cannula placement was verified. Pups from 6 litters were tested (n = 12 pups per group). A two-way, repeated measures ANOVA (Trials X Condition) was performed on 3 minute blocks of data. Post-hoc comparisons of differences between individual treatment groups were performed using Tukey's HSD test.

Results and Discussion

During the first part of testing, the behavior of pups in the Oral/Oral group (Figure 3) was similar to the patterns of behavior of 6-hr deprived 18-day-olds described in Experiment 1 (Figure 1C). Thus, the presence of the intragastric cannula did not appear to affect pups' responsiveness. Pups in the Gastric/Oral and No Infusion/Oral groups showed very few mouthing responses during the first half of testing. In the second part of testing, the

responses of pups in the Oral/Oral group were different than the responses of both Gastric/Oral and No Infusion/Oral pups (Condition, $F[2, 15] = 11.3$, $p < 0.001$). The responding of Gastric/Oral pups was similar to the responding of No Infusion/Oral pups in all blocks of trials except one; during the 14th trial block, Gastric/Oral pups mouthed more than No Infusion/Oral pups ($p < 0.05$). The responsiveness of all pups declined during testing (Trials; $F[19, 285] = 38.33$, $p < 0.0001$).

These results demonstrate that the decrement in responding to repeated oral sucrose infusions is not due to the accumulation of gastric and post-gastric signals since direct intragastric infusions fail to produce any decrement in oral responsiveness. Note that, while the gastric infused group may have received the greatest gastric and post-gastric stimulation because orally infused pups tended not to consume a portion of the final infusions, the mouthing responses of Gastric/Oral pups was significantly higher even during the last block of infusions.

GENERAL DISCUSSION

A habituation-like process. Repeated oral exposure to a diet stimulus results in a decline in ingestive behavior in rat pups. This decrement is not due to gastrointestinal signals, post-absorptive signals, or fatigue because merely altering the oral properties of the diet partially restores responsiveness and intragastric infusions fail to reduce mouthing responses. These results suggest that the decline in feeding behavior is due to experience and can be conceptualized as a habituation of orosensory and/or oromotor responsiveness. An advantage of the habituation concept is that it casts feeding phenomena (previously termed sensory- or stimulus-specific satiety) in a broader framework of well explored analytic approaches and strategies. Further, an explanation in terms of habituation calls explicit attention to parameters of oral responsiveness which may be modulated during normal

feeding.

Given the small amount of diet actually infused, a pup's inability to digest and absorb it, and the complete lack of independent effects of the diet as seen in Experiment 3, the decline in mouthing responsiveness seen in pups of all ages and most deprivation conditions by the end of testing is quite remarkable. Pups of these ages are known to be willing to consume much larger volumes of diet than those actually infused here (e. g. Hall and Bryan, 1980), but here, they all stop responding and let much of the final infusions spill from their mouths. This potent inhibition of ingestive responsiveness probably resulted from the intermittent but repetitive nature of the small infusions. This repeated stimulation was explicitly intended to assess oral experience effects, rather than produce post-ingestive feedback, and represents a procedure like that typically used to evaluate habituation. The nature of this stimulation procedure could have contributed to the detection of a strong experiential inhibition in a number of ways.

First note that while earlier studies assessed intake, our tests measured oral responsiveness. Perhaps a pup's willingness to continue mouthing does not reflect its willingness to actually consume the diet. However, in previous studies of pups' mouthing behavior during both real and sham feeding there was a correlation between the amount of mouthing behavior and amount of diet consumed. Various other procedures used to assess an animal's oral responsiveness (e.g. taste reactivity tests) have demonstrated similarities between intake and responsivity (Flynn & Grill, 1988; although see Pelchat, Grill, Rozin & Jacobs, 1983). Therefore, while the chance that oral responsiveness and actual ingestion are unrelated is remote, it is a possibility which deserves further examination.

A second possibility is that the effects of experience increase in importance as oral experience is spread out in time. In previous studies, larger quantities of diet were infused more rapidly, or pups were allowed to consume the diet, quickly and continuously, directly

from the floor of test containers. Thus the majority of intake in these studies may have occurred before habituation had a chance to develop.

A third and more interesting possibility is that the present procedure exposes a dynamic interaction between oral habituation and sensory adaptation processes. Normal ingestion (and previous test procedures) allow diet to remain in contact with a pup's mouth for extended periods of time, and during such lengthy contact with a diet, peripheral sensory receptor adaptation is likely to occur. If sensory adaptation at a peripheral level occurs, those more central levels of processing which involve habituation may be curtailed, since input from the peripheral receptors has been reduced. Processes such as habituation normally may only emerge as contact with the diet becomes less prolonged -- as the animal pauses in its eating behavior, thereby avoiding the emergence of sensory adaptation. In the present study, because pauses were explicitly imposed by presenting brief stimuli with relatively long intervals between stimuli, peripheral adaptation of sensory receptors was reduced to emphasize habituation and the potential effects of oral experience.

If habituation is responsible for decremented responding, why is the response not fully restored when a new diet is introduced? The most likely explanation is that pups generalize between the two flavors - either because their taste systems are not fully developed or because the added flavors are relatively weak stimuli compared to the taste of the sucrose. Substitution of a second diet which differed from the first diet in more than one dimension (i.e., texture, smell, taste, temperature) might be expected to enhance the recovery of responding.

Developmental change in the importance of habituation. A second striking feature of the present results is that the pattern of responding depends on both the length of deprivation prior to testing and on the age of the animal. These altered effects of

deprivation over the preweaning period may reflect a developmental change in the significance of habituation. Since habituation is most robust (i.e. least affected by deprivation) in older pups, we may be observing the emergence of an important mechanism in the control of normal feeding in both preweaning and adult animals.

The occurrence of habituation was most affected by the length of prior deprivation in 6-day-old pups. Less striking deprivation effects were observed by 12 days of age and by 18 days of age, no influence of deprivation on habituation patterns is evident. Animals in all 18-day-old groups showed similar levels of responding throughout testing with marked decreases in mouthing by the end of testing. This diminishing impact of deprivation is especially intriguing given the distinct effect of deprivation on intake -- intake is similarly increased by deprivation in pups at all these ages (e. g. Hall, 1979; Hall & Bryan, 1980). If anything, one might have expected that as pups approach weaning age their willingness to ingest, here indexed by the duration of mouthing, would become more responsive to manipulations such as deprivation.

One possible explanation for the differences in habituation patterns in pups at the three ages is that nutrient deprivation conditions may not be comparable following similar deprivation lengths. The state of a 6-day-old after 24 hrs deprivation may not be the same as the state of a 24-hr deprived 18-day-old. This seems unlikely for several reasons. First, 6-day-old pups appear to be insensitive to the nutritive aspects of deprivation (Hall, 1990). Second, previous results have shown that pups at these ages lose similar percentages of their body weight during similar lengths of deprivation (Hall, 1979). Nevertheless, the possibility that increasing the deprivation period for older pups might affect patterns of habituation cannot be ruled out.

This shift in habituation patterns in response to deprivation does occur within the developmental time frame during which a number of other controls of ingestion emerge. For

example, in 6-day-old pups dehydration is the most pronounced stimulus to ingest and level of gastric fill is a potent brake on intake. Pups at 12 days of age have developed an additional, nutritive, suppressive control but their intake remains responsive to hydrational status (Swithers & Hall, 1989). By 18 days of age, the influence of hydrational status is considerably reduced and ingestion is governed by factors more closely related to metabolic status. Thus, the independent ingestion of 6-day-olds appears most closely associated with adult drinking behavior, while ingestion of 18-day-olds appears to be more like adult feeding behavior. Perhaps issues related to hydrational status affect habituation of mouthing responses.

In summary, we have demonstrated that oromotor experience within an ingestive episode can influence the pattern of ingestive behavior during that episode and that these effects of oromotor experience are specific to the diet which is consumed. The role of this experience depends on both the physiological state of the animal and its developmental age. Habituation of orosensory and/or oromotor responsiveness appears an appropriate conceptualization of the decline in oromotor responsiveness in the present study as well as more generally for processes termed stimulus-specific or sensory-specific satiety.

Figure Captions

Figure 1.

A. Patterns of mouthing behavior in response to sucrose infusions for 6-day-old pups in all deprivation conditions over the course of 21 trials. Each point represents the average (\pm SEM) number of seconds of mouthing per trial, averaged over 3 trial blocks.

* $p < 0.05$ compared to non-deprived pups.

B. Patterns of mouthing in 12-day-old pups.

C. Patterns of mouthing in 18-day-old pups.

Figure 2.

A. Partial restoration of mouthing responses of 6-day-old pups after switching diet flavor, points are number of seconds mouthing per minute averaged over 3 trial blocks. Pups in the Same condition received the same flavor throughout testing; pups in the Switch condition received one flavor in Blocks 1-5 and a second flavor in Blocks 6-10.

* $p < 0.05$

** $p < 0.01$

B. Patterns of mouthing responses to two flavors of sucrose solution in 12-day-old pups. Flavors were switched between blocks 7 and 8.

C. Patterns of mouthing in 18-day-old pups. Flavors were switched between blocks 7 and 8.

Figure 3. Patterns of mouthing in pups receiving an oral infusion, gastric infusion or no infusion for the first 10 blocks of 3 trials. All pups received oral infusions in Blocks 11-17. See text for explanation of legends.

* $p < 0.05$ compared to Oral/Oral group

Figure 1

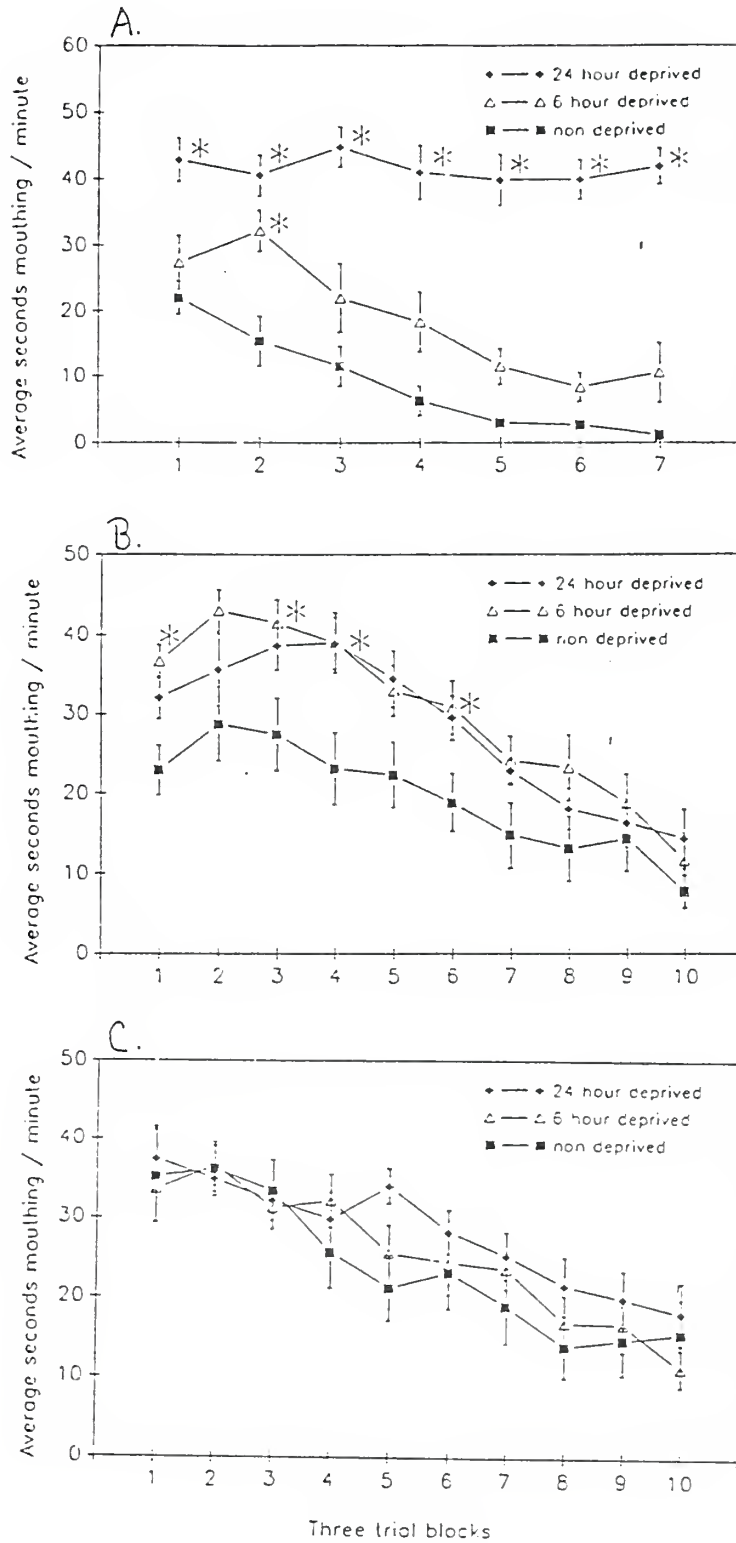


Figure 2

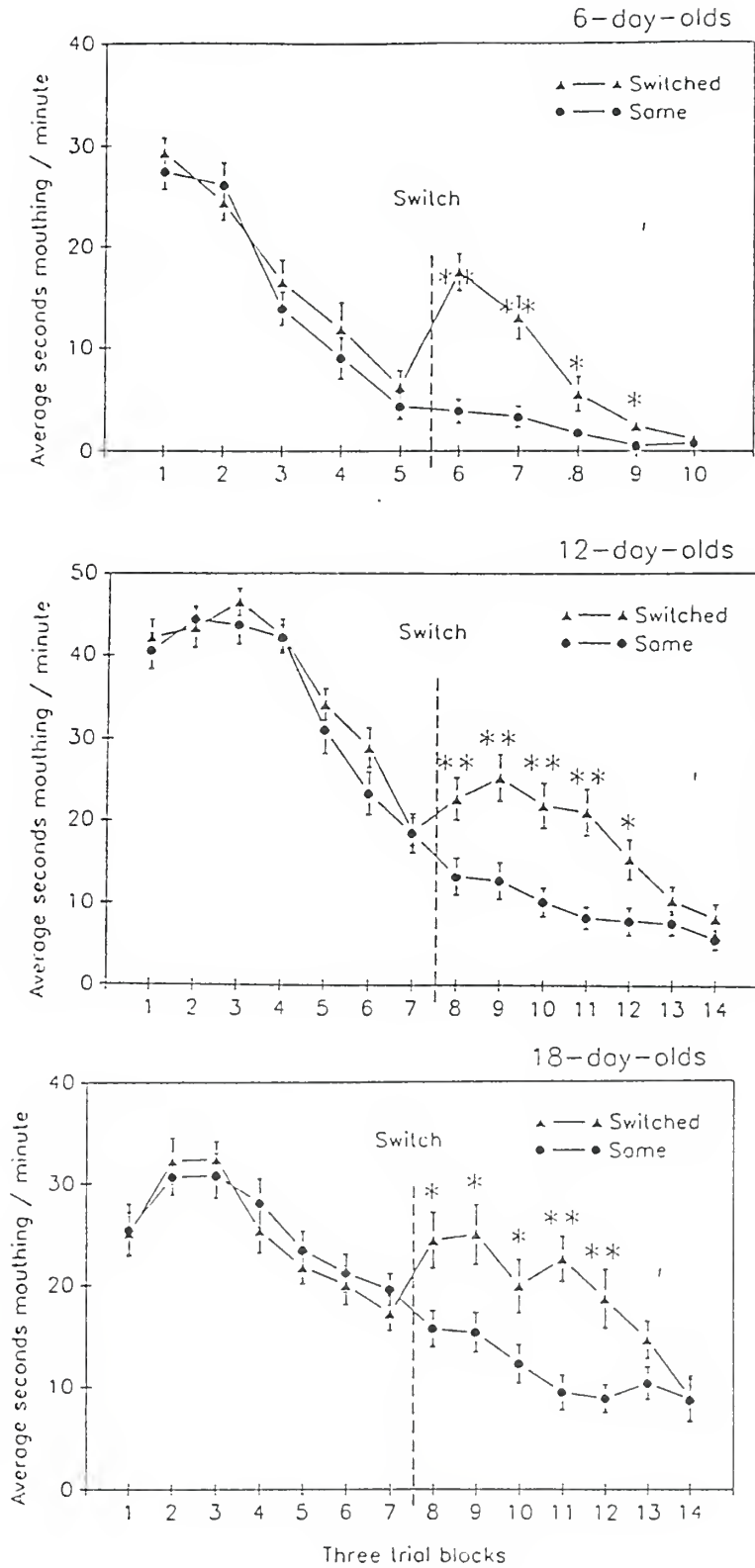
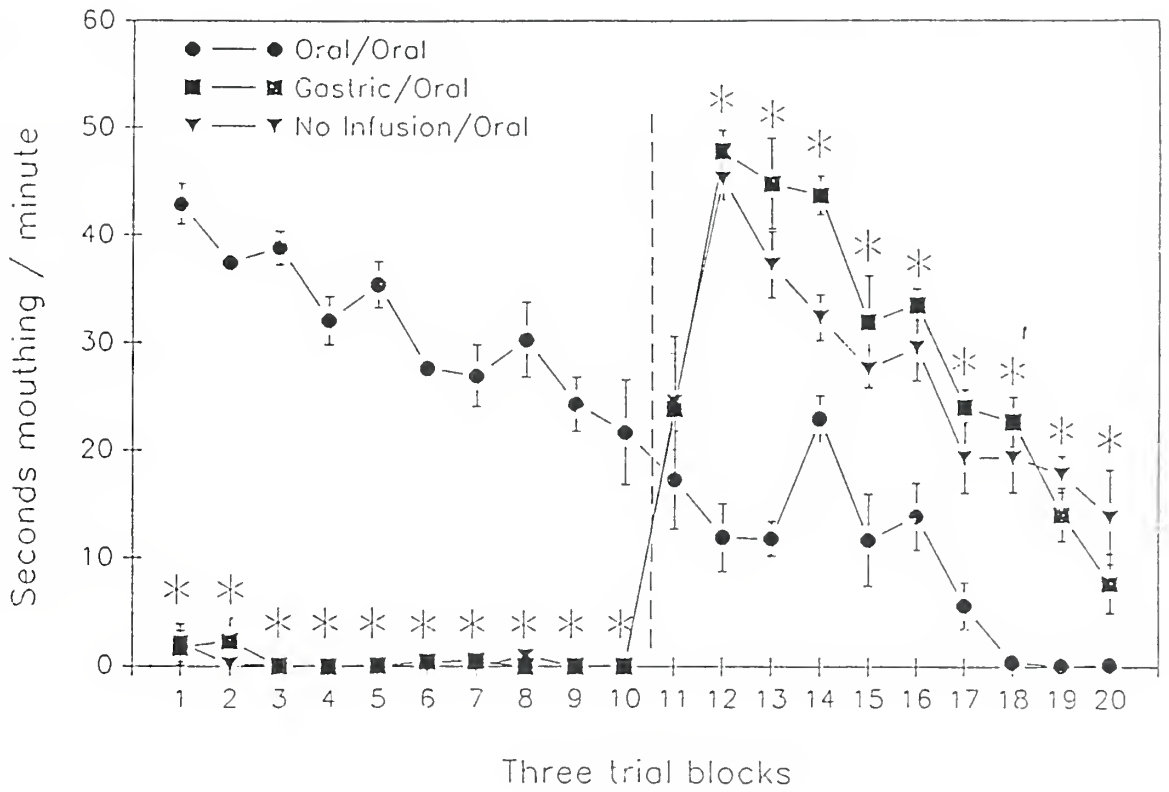


Figure 3



CHAPTER II.

CONTROL OF INGESTION BY ORAL HABITUATION IN RAT PUPS

ABSTRACT

The role of habituation of mouthing activity in the control of ingestion was investigated in 6-, 12-, and 18-day-old rat pups. In pups at all ages, oral habituation to a flavored diet inhibited ingestion of a continuous oral infusion of that same diet. Twelve-day-old pups that had orally habituated to a diet continued to consume less of a continuous oral infusion of that diet both 30 minutes and 3 hrs later and the duration of suppressed ingestion was shown to be dependent on the rate of stimulus presentation during habituation experience. These data suggest that oral habituation may be a diet-specific influence on both intra- and inter-meal patterning.

INTRODUCTION

Oral experiences that are generated by the act of ingestion have long been assumed to contribute to the control of ingestive behavior (e.g. Kohn, 1951). Prominent among the effects of such oral experience, and one of the most direct contributions that sensorimotor experience in any domain can make, is the immediate diminution of the ongoing response based on recent stimulation or activation of the response. Stated in another manner, oral responses can habituate with use and the degree to which they habituate influences their subsequent expression. Although the role of the mouth is not usually viewed by psychobiologists from a habituation perspective (though see Thorpe, 1966, and other ethological perspectives, e.g. Tinbergen, 1951) the concept of habituation provides a way of understanding what is meant by the "oral phase" or "oral factor" in feeding control, a control also frequently termed "oral metering".

We have previously shown that the responsiveness of the oral consummatory component in rat pups is dependent on its prior expression; in response to repeated brief intra-oral infusions of sucrose solution, oral activity declines (Swithers-Mulvey, Miller & Hall, 1991). That this decline was not due to post-ingestive consequences was demonstrated in the following ways: 1) by restricting the amount of diet infused to a small total volume; 2) by using sucrose, a sugar which young rats do not readily digest or absorb (e.g. Henning, 1987); and 3) by demonstrating that direct intragastric infusions alone failed to affect oral responsiveness. The decline in responsiveness was not due to fatigue because simply changing the flavor of the infused diet elicited an increase in mouthing response levels. In addition, this use-dependent oral ingestive control showed age-related differences in response to deprivation. In 6-day-old pups, lengthy deprivation periods attenuated the habituation to oral infusions. In contrast, 18-day olds exhibited a marked decline in oral response to intra-oral infusions even following 24 hrs of deprivation. Thus, oral experience

can significantly modulate the further expression of the oral consummatory component. This decline in oral responsiveness to repeated stimulation resembles habituation as recognized and described in a number of other response systems, from response systems of invertebrates to response systems of humans. This oral habituation appears to be a strong potential modulator of the expression of the consummatory response to repeated stimulation.

What is unexplored, however, is how this experience-based effect influences subsequent ingestion and how persistent such influences may be. The aims of the present study were to examine how oral habituation to repeated stimulation affected an animal's actual intake in a feeding test, to investigate the duration of such an effect and to determine whether the effects of oral habituation on intake change during development. Pups at 6, 12 or 18 days of age received a habituation experience during which repeated intra-oral infusions of a flavored diet were made. Following this habituation experience, a number of features of actual consumption were examined. In Experiment 1, intake of a diet infused continuously into the pups' mouths was measured. In a second experiment, the effects of habituation experience on intake were assessed during an ingestive test in which pups consumed diet from the floor of test containers instead of consuming intra-oral infusions. In Experiment 3, the duration of the effects of oral habituation on intake was measured by imposing delays of 0, 30 or 180 min between the habituation experience and the intake test. Finally, in Experiment 4, the effects of changing the rate of stimulus presentation during habituation experience on the pattern of oral habituation and the duration of its effects were examined.

GENERAL METHOD

Subjects

Subjects were progeny of Charles River CD strain rats maintained in the breeding

colony in our laboratory. After mating, pregnant females were housed individually in polyethylene cages during the week prior to parturition. Cages were checked for births daily between 0800 and 1700 hrs and pups found before 1700 hrs were considered 0 days of age. Litters were culled to 10 pups (5 male and 5 female when possible) at 1 day of age. The colony room was maintained at 21-23 °C (40-70% relative humidity) on a 14:10 light:dark cycle.

Cannula placement

Six hrs prior to testing, pups were removed from the mother's cage and oral and gastric cannulas were implanted under methoxyflurane (Metofane, Pitman-Moore) anesthesia as previously described (Hall, 1979; Phifer & Hall, 1987). Briefly, anterior oral cannulas were constructed from a 10 cm length of polyethylene tubing (PE-10), flanged on one end and fitted to one end of a curved piece of stainless steel wire. Cannulas were then implanted by inserting the wire through the soft floor of the oral cavity just posterior to the lower incisors. The tubing was drawn through the lower jaw until the flanged end was seated against the inner surface of the mouth. This placement permitted the pup to freely ingest or reject infused diets.

Gastric cannulas were implanted by passing a guide tube (made of soft rubber Silastic tubing for 6- and 12-day-old pups and of 27 gauge syringe tubing with a Silastic tip for 18-day-old pups) down the esophagus and into the stomach. One end of a stainless steel wire was inserted through the guide tube and out through the wall of the stomach and left abdominal wall. The guide tube was then withdrawn from the pup's mouth. A 30 cm length of PE-10 tubing with one end flanged by heating was friction fitted to the end of the wire extending from the pup's mouth. The wire and attached tubing were then gently pulled through the stomach and abdominal wall until the flange rested against the inside surface of the stomach. Following cannula placement, all pups were housed in plastic containers lined

with absorbent bedding inside warm, humid incubators until the time of testing.

Habituation experience

For clarity, the initial phase of testing will be termed 'habituation experience'.

Immediately prior to habituation experience, pups were stimulated to urinate and defecate by stroking their anogenital region with a soft brush. Pups were then weighed to the nearest 0.01 g and placed into clear plastic test containers (6.5 x 11 x 12.5 cm) inside a warm, humid glass incubator. The oral and gastric cannulas were attached to lengths of plastic tubing which were connected to 5cc syringes mounted in a Harvard infusion pump. A programmable timer controlled the action of the infusion pump. Pups were allowed a 15 minute accommodation period in the testing containers, with the cannula leads attached, prior to the habituation experience. During habituation experience, brief (3 sec) infusions of a solution were delivered once every minute (except where noted in Experiment 4) through the cannulas; pups' mouthing behaviors were scored continuously. Whenever possible, observers were blind to pups' conditions. Mirrors behind and under the clear sides of the test containers allowed easy viewing of pups' mouthing activity. "Mouthing" activity was defined as opening or closing of the jaws and/or movement of the tongue and was recorded second by second.

Statistical analysis

In each experiment, separate two-way repeated measures ANOVAs were performed (one at each age in Experiment 1) on the weight gain during habituation experience and intake during the consumption test. In Experiment 4, a two-way repeated measures ANOVA was also performed on mouthing scores (PC-SAS, release 6.03, Statistical Analysis Systems, Cary, NC). Duncan's Multiple Range Test was used for post-hoc comparisons between different conditions within each age, with a $p < 0.05$ taken as significant.

EXPERIMENT 1: DEVELOPMENTAL EFFECTS OF ORAL HABITUATION ON INGESTION

Previous studies (e.g. Swithers-Mulvey et al., 1991) have demonstrated that the oral activity of rat pups diminishes after repeated oral stimulation. This oral habituation is marked, diet specific, and affected in an age-related manner by prior deprivation. To determine whether this diminished oral activity can influence subsequent actual ingestion, consumption of a continuous oral infusion of diet was measured following oral habituation in pups at developmental ages spanning the preweaning period.

Procedures

Pups were tested once only at 6, 12, or 18 days of age. At each age, during the initial habituation experience, two groups of pups received a series of 30 oral infusions of 3 sec duration of one of two flavored diets (0.1% Grape or Cherry Kool-Aid in 5% (w/v) sucrose). Two control groups received an identical paired series of infusions of one of the flavored diets intragastrically. A final control group received no infusions (See Table 1 for Experimental Design). At each age, two pups (one male, one female) from each of six litters were tested in each condition ($n = 11$ -12 pups at each age in each condition). Immediately following habituation experience, pups were reweighed and returned to test containers. Because the 18-day-old pups appeared more reactive to the handling required to remove them from the test containers, pups at this age remained in test containers throughout habituation experience, consumption testing and weighing. These test containers were constructed with wire mesh flooring so that any diet the pups did not ingest spilled out of the container.

During the consumption test, all pups received a 4 minute continuous oral infusion of one of the flavored diets. One group of pups that previously received oral infusions received the same flavor during habituation experience and consumption testing while the other group

was switched to the different flavor. Likewise, one of the two gastric groups received oral infusions of the same flavor that it had previously received intragastrically while the second gastric group was switched to the different flavor. Pups in the non-infused group received one of the two flavors at random. Following this 4 minute infusion, pups (or for 18-day olds, pups and containers) were reweighed and weight gain (expressed as percentage of pre-habituation experience body weight) was used to determine intake.

Results and Discussion

Repeated oral stimulation during habituation experience produced decreases in mouthing activity in pups of all ages (Figures 1 A-C) consistent with previous findings (Swithers-Mulvey et al., 1991). More importantly, results of the 4 minute consumption test (Figure 2) indicate that habituation of oral activity reduced subsequent intake in pups of all ages (Main effect of Consumption Test Condition; 6-day olds, $F[4, 49] = 9.04$, $p < 0.0001$; 12-day olds, $F[4, 52] = 12.78$, $p < 0.0001$; 18-day olds, $F[4, 51] = 8.74$, $p < 0.0001$).

Six-day-old pups that had habituated to a diet consumed less of the subsequent diet than either intragastrically or non-infused pups (Figure 2A). Suppression of intake was not diet-specific at this age; there were no differences between the intake of Oral/Same and Oral/Switch pups. Gastric infusions of the same or different flavor had no effect on consumption in 6-day-olds, since their intake did not differ from non-infused (Group None) controls.

In 12-day-old pups, oral habituation affected intake in a diet-specific manner; pups in group Oral/Same consumed less than all other groups (Figure 2B). This oral specificity was not due to Oral/Same pups having consumed more than Oral/Switch pups during habituation (Table 2). In 12-day-old pups, groups Oral/Switch, Gastric/Same and Gastric/Switch also ingested significantly less than non-infused pups.

In 18-day olds, only pups in group Oral/Same consumed significantly less than non-

infused pups (Figure 2C). Gastric infusions and infusions of a different flavored diet had no effect on consumption compared to no infusions.

These results indicate that oral habituation to a diet has powerful suppressive effects on the amount of that diet actually ingested. In very young pups, this suppressive effect is generalized to a diet with a different flavor. This lack of specificity may be partly attributable to the immature sensory systems of such young pups and to the similarity of the two flavored diets. By 12 days of age, ingestion of a flavored diet is decreased significantly more by previous habituation experience with that flavor; habituation to a different flavor has smaller effects on subsequent intake. This specificity is not post-ingestive, since no differences in amount consumed were seen between groups which received the same or different flavors intragastrically. At 12 days of age, ingestion was partially inhibited by gastric infusions or oral infusions of a different diet, while at 6 or 18 days of age gastric infusions had no effect. This inhibition of ingestion in 12-day-old pups is likely due to the gastric signals related to fill accruing from the infusions during habituation. As seen in Table 2, 12-day-old pups had received a larger proportion of their body weight during habituation compared to 6- and 18-day olds.¹ Nevertheless, the most profound reduction was noted in pups following oral habituation to the same flavor (Figure 2B). By 18 days of age, it is clear that oral habituation to a diet powerfully and specifically inhibits ingestion of that diet (Figure 2C). This potent effect is due to oral experience and not post-ingestive effects of the stimulation.

This experiment demonstrates that when the pup is required to produce only the final oral consummatory component during habituation and consumption, intake is inhibited by oral habituation. It is possible, however, that this suppressed intake is expressed only

¹The increase in body weight gain during habituation in 12-day olds is at least partly due to pups being somewhat lighter at the time of testing than is average for our lab.

because the habituation and consumption experiences are similar in that in both situations diet is delivered directly into the pup's mouth through an oral cannula. The second experiment thus examined the effects of oral habituation in a different ingestive situation.

EXPERIMENT 2: EFFECTS OF ORAL HABITUATION ON INGESTING FROM THE FLOOR

During normal ingestion, animals must exhibit more than the final consummatory component of ingestion in order to actually ingest. Inhibition of the final component following oral habituation might be expected to translate to inhibition of the entire ingestive sequence. However, the effects of some manipulations (e.g. Broder, Smith, Tyrka & Gibbs, 1990; Tyrka, Smith & Gibbs, 1990) in tests of intake which examine only the oral consummatory component are not identical to the effects in tests of the entire ingestive sequence. Thus, when a pup controls the initiation and maintenance of contact with the diet and therefore its introduction into the mouth, the experience of the prior direct intra-oral infusions may become less relevant. This experiment was designed to determine whether oral habituation would suppress ingestion in a test requiring more of the behavioral components of ingestion and thus more closely approximating the normal ingestive situation in adult animals.

Procedures

Pups were tested at 12 days of age. During habituation experience, two groups of pups received a series of oral infusions of one of two flavored sucrose solutions as described in Experiment 1. A third group received identical paired infusions through their gastric cannulas and a final group received no infusions (see Table 3 for Experimental Design). In each condition, one male and one female pup from each of 6 litters were tested (n=11-12 pups in each condition). Immediately following habituation experience, pups were reweighed and placed into new test containers. On the floors of these containers were

paper towels wetted with one of the two flavored diets. Thus, in order to ingest the diet pups had to contact and actively lick and lap the diet up from the floor. One group of pups which had previously received oral infusions was placed into containers with the same flavor solution as during habituation experience, while the second group of orally-infused pups were switched to the different flavor diet. The gastric and non-infused pups were given one of the two flavors at random. Pups were allowed to ingest the diet from the floor of these test containers for 21 minutes. Pups were then reweighed, and weight gain (expressed as a percentage of pre-habituation experience body weight) was used to measure intake.

Results and Discussion

As seen above, mouthing activity to repeated oral infusions habituated (Figure 3A). This oral habituation to a diet had clear suppressive effects on the amount of that same diet consumed in a test of ingesting from the floor (Figure 3B; Main effect of Consumption Test Condition; $F[3, 44] = 7.77$; $p < 0.001$). The suppressive effect of oral stimulation was specific to the diet experienced orally; pups ingesting a flavor different from the one to which they had orally habituated consumed the same amount as intragastrically or non-infused controls. This diet-specific suppression was not due to pups in the two oral conditions consuming different amounts during habituation experience since weight gain during habituation was similar in pups in both oral conditions.

These results further demonstrate that habituation of mouthing following repeated oral stimulation reduces subsequent intake. This suppression of intake is noted even in a different ingestive situation in which pups are required to express a number of components in addition to the final oral consummatory component.

EXPERIMENT 3: DURATION OF SUPPRESSED INGESTION BY ORAL HABITUATION

Oral habituation in pups 12 days of age and older thus appears a potent, specific

and immediate control of within-bout ingestive behavior. A second potential contribution of oral habituation is to influence the duration of the intake terminating or satiety process and to thus influence inter-bout intervals. If oral habituation is important to intake control and the overall patterning of ingestive bouts, the suppressive effects of oral habituation must have more than a momentary duration. This experiment examined the duration of suppressed ingestion following oral habituation.

Procedures

Pups were tested once only at 12 days of age. In each condition, 1 pup from each of 8 litters was tested ($n=8$ pups in each condition). During habituation experience, two pups in each of three groups received a series of oral infusions of a flavored saccharin solution (0.1% grape or cherry Kool-Aid in 0.05% saccharin). A third pup in each group received no infusion (see Table 4 for Experimental Design). Saccharin was used in this experiment to extend the habituation analysis to another stimulus, and because of concern about the longer-term effects of the non-absorption of sucrose solutions. A concentration of 0.05% saccharin was chosen because it elicited mouthing durations similar to 5% sucrose in pilot experiments. Following habituation experience, intake of a 4 minute continuous infusion was determined at one of three retention times: immediately following habituation experience (as in Experiment 2); 30 minutes after the end of habituation experience; or 3 hrs after the end of habituation experience. During the retention period, all pups, including control pups, remained in their test containers inside the incubator. Immediately prior to the intake tests, pups were weighed and weight gain (expressed as a percentage of the respective control pup's weight gain) during the continuous infusion was used to determine intake.

Results and Discussion

As seen with oral habituation to sucrose immediately following habituation, intake of

pups that had orally habituated to a flavored saccharin solution was significantly lower than intake of pups that had habituated to a different flavored diet or pups that received no infusions (Figure 4). When intake was assessed 30 minutes following habituation experience, intake was still lower in Oral/Same pups compared to Oral/Switch and None pups. Three hrs after oral habituation to a flavored diet, Oral/Same pups continued to ingest less of that diet than pups that received no infusions; however, after a 3 hr delay, intake of the same flavored diet was similar to intake of a different flavored diet (Figure 4; Main effect of Consumption Test Condition; $F[8, 63] = 8.33, p < 0.001$).

These results indicate that oral habituation has a potent, diet-specific, immediate effect on intake, and that this specific suppression of consumption lasts at least 30 minutes. Following a delay of 3 hrs, intake is still suppressed compared to non-infused pups, but has become less specific since intake of the same flavor was similar to intake of a different flavor. This duration of effect appears consistent with a potential role for oral habituation in the patterning of not only of intra-meal intervals but also in longer term, inter-meal intervals (e.g. Le Magnen & Devos, 1980).

EXPERIMENT 4: EFFECTS OF STIMULUS TIMING

In the experiments described above, intake is suppressed at least 30 minutes following a habituation experience during which pups received brief (3 sec), discrete infusions of solutions separated by relatively long inter-stimulus intervals (ISI's). However, during normal ingestion, the timing of stimulus presentation is at the animal's control and therefore ISI's are considerably more variable and often substantially shorter (e.g. Davis, 1989) than our arbitrarily chosen one minute interval. In a number of studies of habituation in other response systems, the timing of stimulus presentation during habituation has been found to influence both rate of habituation and duration of habituated responding (Davis,

1970). Thus, in the present experiment we examined whether changes in the timing of stimulus presentation influence the expression of the decremented responding and/or the duration of habituation's suppressive effect on intake.

Procedures

Pups were tested once at 12 days of age. During habituation experience, two pups in each of two groups received 30 oral infusions of a flavored saccharin solution (0.1% Grape or Cherry Kool-Aid in 0.05% saccharin) each of 3 sec duration. In one group, these infusions were delivered once every minute, as in the above experiments. In the second group these infusions were delivered once every 20 seconds. A third pup in each group received no infusions (see Table 5 for Experimental Design). Intake was assessed separately for each group during a 4 minute continuous infusion test given 30 minutes following the end of their respective habituation experience. In each condition, one pup from each of 8 litters was tested.

Results and Discussion

Patterns of decreased oral responding were similar in both groups (Figure 5A; note that duration of mouthing is expressed as a percentage of the ISI and not as seconds mouthing; Main effect of Trials, $F[9, 252] = 45.42, p < 0.001$; Main effect of Interval Condition, $F[3, 28] = 1.75, n.s.$). However, when intake was tested 30 minutes following habituation, ISI had a marked effect (Figure 5B; Main effect of Interval Condition, $F[5, 42] = 13.83, p < 0.001$). As in Experiment 4, pups receiving a one minute ISI demonstrated suppressed intake of the same diet experienced orally while intake of a different flavored diet was not suppressed. In contrast, pups receiving the oral infusions once every 20 seconds showed no evidence of suppressed ingestion. These results demonstrate a profound influence of rate of stimulus presentation on duration of the effects of oral habituation. The effects of rapid stimulus presentation (20-sec ISI) are short-lived, while a

longer interval between oral infusions (1-minute ISI) produces sustained decrements in ingestive responding. These effects of timing are noteworthy given the standard methods of looking at feeding control in which timing of stimulus presentation is at the animal's discretion.

GENERAL DISCUSSION

Control of ingestive behaviors is undoubtedly accomplished by complex interactions among central and peripheral processes. One approach to understanding control of such complicated interactions is to recognize that ingestion is not a singular behavior, but instead is a sequence of closely related component behaviors (e.g. Hall, 1990). Even the most simple description of these component behaviors would include an activation/arousal component, an orienting/search component and a final oral consummatory component. Acknowledgment of the component nature of ingestive behaviors leads to awareness that each component behavior may be separately modulated and neurally represented. Thus a first approach to discerning how ingestion is controlled is to examine the control of individual component behaviors in isolation from one another. Such isolation is readily accomplished for at least one ingestive component - the final oral consummatory component - by simple, direct intra-oral infusion of diets.

In fact, dissociation of the oral consummatory component from the preceding appetitive components indicates that the oral consummatory component itself may exert an important influence in ingestive control. We have shown here (and previously; Swithers-Mulvey et al., 1991) that in response to repeated stimulation, oral responsiveness declines. This diminution of oral responsiveness to repeated oral infusions of a diet is remarkably robust. This decline is noted in the youngest animals tested and is expressed in older animals even following lengthy deprivation periods (Swithers-Mulvey et al., 1991). The

results of the present study demonstrate that this oral habituation process can be a relevant control of actual ingestive behavior.

Even in the youngest pups tested, oral habituation to a diet caused a significant suppression of actual intake in a direct infusion test. The specificity of these suppressive effects on ingestion was not fully expressed in the youngest pups; intake of even a different flavored diet was low following habituation in 6-day olds. This lack of specificity is probably not a developmental effect on habituation per se, but is instead related to the immaturity of sensory systems in pups of this age and to the similarity of the two flavored diets. Intake of two more distinct diets might be more specifically suppressed even in very young animals.

The effects of having orally experienced the diet were striking when compared to the effects of having the same amount of diet infused directly into the stomach. In 6- and 18-day-old pups, intragastric infusions did not affect intake. In 12-day-old pups, gastric infusions themselves suppressed intake, but this suppression of intake by gastric infusions is not particularly surprising. A number of previous studies (e.g. Deutsch, 1985) have demonstrated that gastric signals alone can affect ingestive behaviors of pups and adult rats. On the other hand, gastric signals alone do not account for all of the effects of oral infusions in 12-day-old pups, since oral stimulation with the same diet suppressed intake significantly more than gastric stimulation alone.

The suppression of intake by an experience-based oral mechanism suggests that oral habituation-like processes may play a role in determining normal intake patterns. This suggestion is strengthened by the observation that significant suppression of intake was seen even when the intake test and the habituation experience were considerably different. Additional support for a role of oral habituation in control of the patterning of ingestion comes from the demonstration that the effects of habituation on intake can be of considerable duration, lasting up to 3 hrs. This duration of effect is consistent with a

mechanism by which both intra- and inter-meal feeding patterns may be established.

Perhaps surprisingly, the duration of effect is highly dependent on the rate of stimulation during the habituation experience. A relatively short ISI produced patterns of mouthing during the habituation experience that are similar to the decrements in mouthing behavior to those produced by a longer ISI. However, a short ISI produced transient effects, while a longer interval produced more lasting effects. The processes by which short and long ISI's produce decreases in mouthing responsivity may represent, respectively, peripheral sensory adaptation and habituation processes. Both of these processes are logically similar, producing a response decrement, yet they differ in the site of action and duration of effect.

The duration of the effects of oral stimulation clearly depends on the rapidity of the presentation of oral stimuli. During a normal ingestive bout, the rate of stimulation is under the animal's control and clearly can be highly variable. Given this variability of stimulus timing during normal ingestion, peripheral adaptation and habituation processes may interact to produce observed patterns of ingestion. Typically, during a normal ingestive bout, the animal has continuous access to the diet and begins ingesting the diet at a rapid rate. During these initial moments of ingesting, the effects of oral experience are likely to resemble the situation of providing stimuli with short ISI's. The diet remains in contact with the sensory receptors and peripheral adaptation potentially develops. This peripheral adaptation then decreases the animal's responsiveness to the diet and the animal pauses in its ingestion, but this effect is short lasting. This pause in ingestion produces an interval that causes the next burst of ingestion to contribute to more central, habituation-like processes. Thus, over the course of an ingestive bout, sensory adaptation and habituation dynamically interact to produce the observed patterning in ingestive responding. In this scenario, the duration of the effects of oral stimulation are then determined by the extent to

which adaptation allows habituation to develop.

In sum, these experiments suggest how a relatively simple oral experience-based decremental mechanism - oral habituation - can exert a potent control over ingestive behavior. By 12 days of age, the effects of oral habituation experience can produce diet-specific reductions in consumption which may last up to 3 hrs. Changing the rate of stimulus presentation underscores the dynamic nature of this oral control of intake and points out the importance of gaining explicit control of the timing of stimulus presentation to the animal in order to understand the range of mechanisms contributing to ingestive control.

TABLE 1: Experimental Design

Experiment 1: Developmental Effects of Oral Habituation on Oral Ingestion

<u>Group</u>	<u>Habituation Experience</u>	<u>Consumption Test</u>
Oral/Same	Flavor A infused orally	Flavor A
Oral/Switch	Flavor B infused orally	Flavor A
Gastric/Same	Flavor A infused gastrically	Flavor A
Gastric/Switch	Flavor B infused gastrically	Flavor A
None	No infusion	Flavor A

During habituation experience, pups received 30 oral or gastric infusions, one every minute, of 3 sec duration. Flavors were 0.1% Grape and 0.1% Cherry Kool-Aid in 5% sucrose. Order of flavor presentation was counterbalanced across litters. During consumption, all pups received a 4 minute continuous oral infusion. Pups were tested once only at 6, 12 or 18 days of age.

TABLE 2: Weight Gain During Habituation Experience (Percent Body Weight \pm SEM)

<u>Group</u>	<u>Age</u>		
	6	12	18
Oral/Same	1.42 \pm 0.21 ^{a,b}	2.64 \pm 0.12 ^a	1.61 \pm 0.25 ^a
Oral/Switch	1.63 \pm 0.08 ^{a,b}	2.42 \pm 0.12 ^{a,b}	1.49 \pm 0.21 ^a
Gastric/Same	2.06 \pm 0.11 ^a	2.73 \pm 0.07 ^a	1.91 \pm 0.17 ^a
Gastric/Switch	1.96 \pm 0.08 ^a	2.80 \pm 0.13 ^a	1.97 \pm 0.15 ^a
None	0.13 \pm 0.03	0.07 \pm 0.05	-0.28 \pm 0.11

Main effect of Consumption Test Condition; 6-day olds, $F[4, 49] = 55.83$, $p < 0.0001$; 12-day olds, $F[4, 52] = 138.13$, $p < 0.0001$; 18-day olds, $F[4, 51] = 24.95$, $p < 0.0001$.

^aSignificantly different from None; $p < 0.05$.

^bSignificantly different from Gastric/Same and Gastric/Switch; $p < 0.05$.

TABLE 3: Experimental Design

Experiment 2: Effects of Oral Habituation on Ingesting from the Floor

<u>Group</u>	<u>Habituation Experience</u>	<u>Consumption Test</u>
Oral/Same	Flavor A infused orally	Flavor A
Oral/Switch	Flavor B infused orally	Flavor A
Gastric	Flavor A or B infused gastrically	Flavor A or B
None	No infusion	Flavor A or B

During habituation experience, pups received 30 oral or gastric infusions, one every minute, of 3 sec duration. Flavors were as in Experiment 1. Order of flavor presentation and flavors delivered gastrically were counterbalanced across litters. Because there were no differences between the Gastric/Same and Gastric/Switch groups in Experiment 1 only one gastric group was tested in Experiment 2. During consumption, pups were allowed to consume diet off the floor of test containers for 21 minutes. Pups were tested once only at 12 days of age.

TABLE 4: Experimental Design

Experiment 3: Duration of Suppressed Ingestion by Oral Habituation

<u>Group</u>	<u>Habituation Experience</u>	<u>Delay</u>	<u>Consumption Test</u>
Immediate			
Oral/Same	Flavor A infused orally	0 minute	Flavor A
Oral/Switch	Flavor B infused orally	0 minute	Flavor A
None	No infusion	0 minute	Flavor A
30 minute			
Oral/Same	Flavor A infused orally	30 minute	Flavor A
Oral/Switch	Flavor B infused orally	30 minute	Flavor A
None	No infusion	30 minute	Flavor A
3 hr			
Oral/Same	Flavor A infused orally	3 hr	Flavor A
Oral/Switch	Flavor B infused orally	3 hr	Flavor A
None	No infusion	3 hr	Flavor A

During habituation experience, pups received 30 oral infusions, one every minute, of 3 sec duration. Flavors were 0.1% Grape and 0.1% Cherry Kool-Aid in 0.05% saccharin. Order of flavor presentation was counterbalanced across litters. Consumption of a 4 minute continuous oral infusion was assessed following a 0 minute, 30 minute or 3 hr delay.

TABLE 5: Experimental Design

Experiment 4: Effects of Stimulus Timing

<u>Group</u>	<u>Habituation Experience</u>	<u>ISI</u>	<u>Delay</u>	<u>Consumption Test</u>

1 Minute				
Same	Flavor A infused orally	1 minute	30 minute	Flavor A
Switch	Flavor B infused orally	1 minute	30 minute	Flavor A
None	No infusion	---	30 minute	Flavor A
20 Second				
Same	Flavor A infused orally	20 second	30 minute	Flavor A
Switch	Flavor B infused orally	20 second	30 minute	Flavor A
None	No infusion	---	30 minute	Flavor A

=====

During habituation experience, pups received 30 oral infusions, one every minute or one every 20 seconds, of 3 sec duration. Flavors were as in Experiment 3. Order of flavor presentation was counterbalanced across litters. Consumption of a 4 minute continuous oral infusion was assessed 30 minutes after the end of habituation experience.

Figure Captions

Figure 1. Patterns of mouthing to a series of oral or gastric infusions of a flavored (0.01% Grape or Cherry Kool-Aid) 5% sucrose solution in A) 6-day-old pups; B) 12-day-old pups; and C) 18-day-old pups. Each point represents the average (\pm SEM) number of seconds mouthing per minute over blocks of three trials. See Table 1 for explanation of legends.

Figure 2. Intake (mean \pm SEM) of a 4 minute continuous oral infusion of a flavored 5% sucrose solution in A) 6-day-old rat pups; B) 12-day-old pups; and C) 18-day-old pups.

Legends as in Figure 1.

* $p < 0.05$ compared to group None

$p < 0.05$ compared to group Oral/Switch

Figure 3.

A. Patterns of mouthing (mean \pm SEM) to a series of infusions of flavored 5% sucrose solution in 12-day-old pups.

B. Intake in 12-day-old rats of a flavored 5% sucrose solution from the floor of test containers during a 21 minute consumption test.

* $p < 0.05$ compared to group None

$p < 0.05$ compared to group Oral/Switch

Figure 4. Intake (mean \pm SEM) in 12-day-old rat pups of a 4 minute continuous infusion of a flavored (0.1% Grape or Cherry Kool-Aid) saccharin (0.05%) solution tested immediately following habituation experience or at a delay of 30 minutes or 3 hrs.

* $p < 0.05$ compared to respective group None

$p < 0.05$ compared to respective group Oral/Switch

Figure 5.

A. Patterns of mouthing in 12-day-old rat pups to a series of oral infusions of a flavored saccharin solution delivered one every minute or one every 20 seconds. Note that each point represents the average number of seconds mouthing expressed as a percentage of the inter-stimulus interval (mean \pm SEM).

B. Intake (mean \pm SEM) in 12-day-old pups of a 4 minute continuous infusion of a flavored saccharin solution 30 minutes after the end of a habituation experience in which the interstimulus interval was 1 minute or 20 seconds.

* $p < 0.05$ compared to respective group None

$p < 0.05$ compared to respective group Oral/Switch

Figure 1.

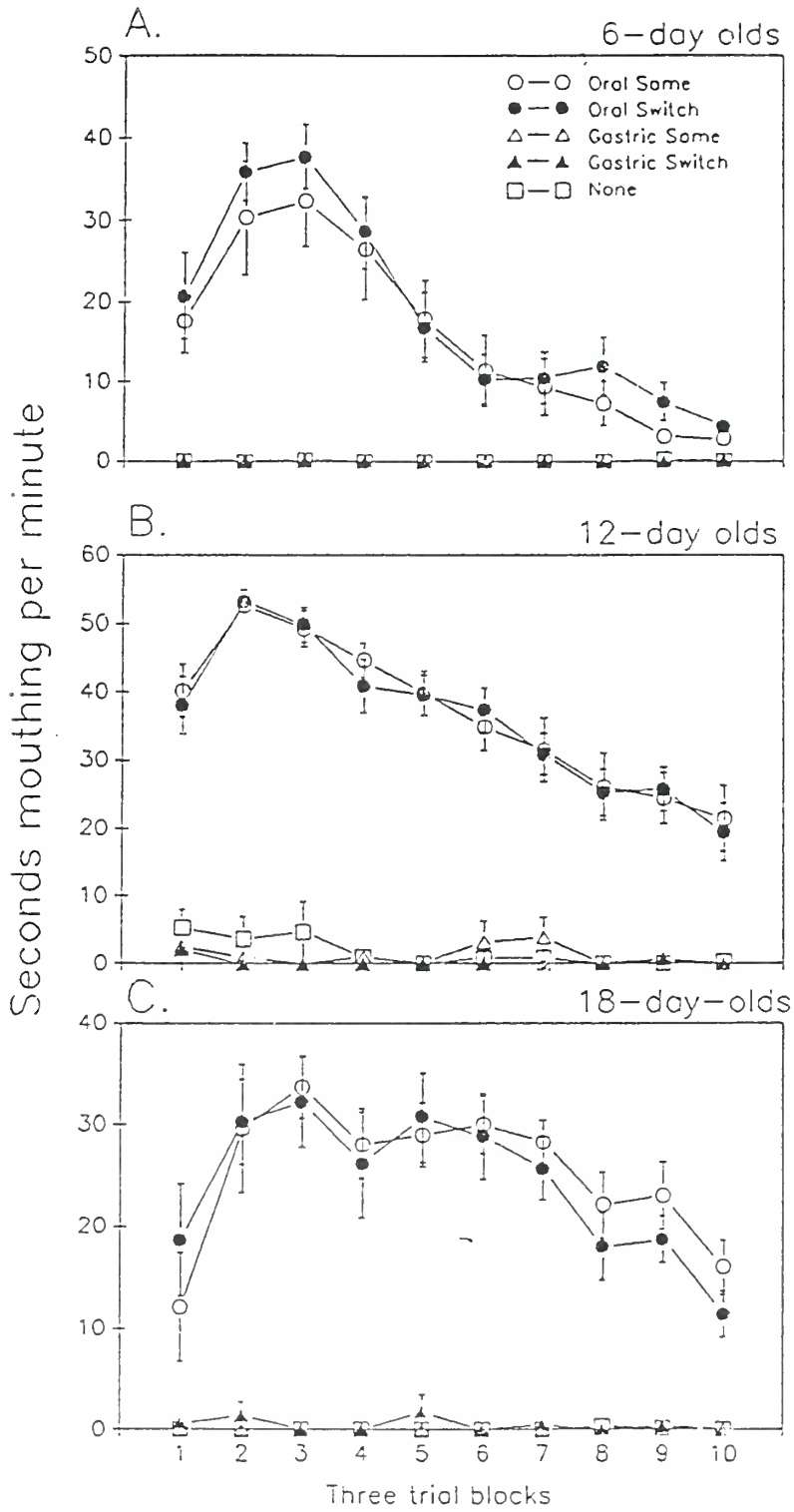


Figure 2.

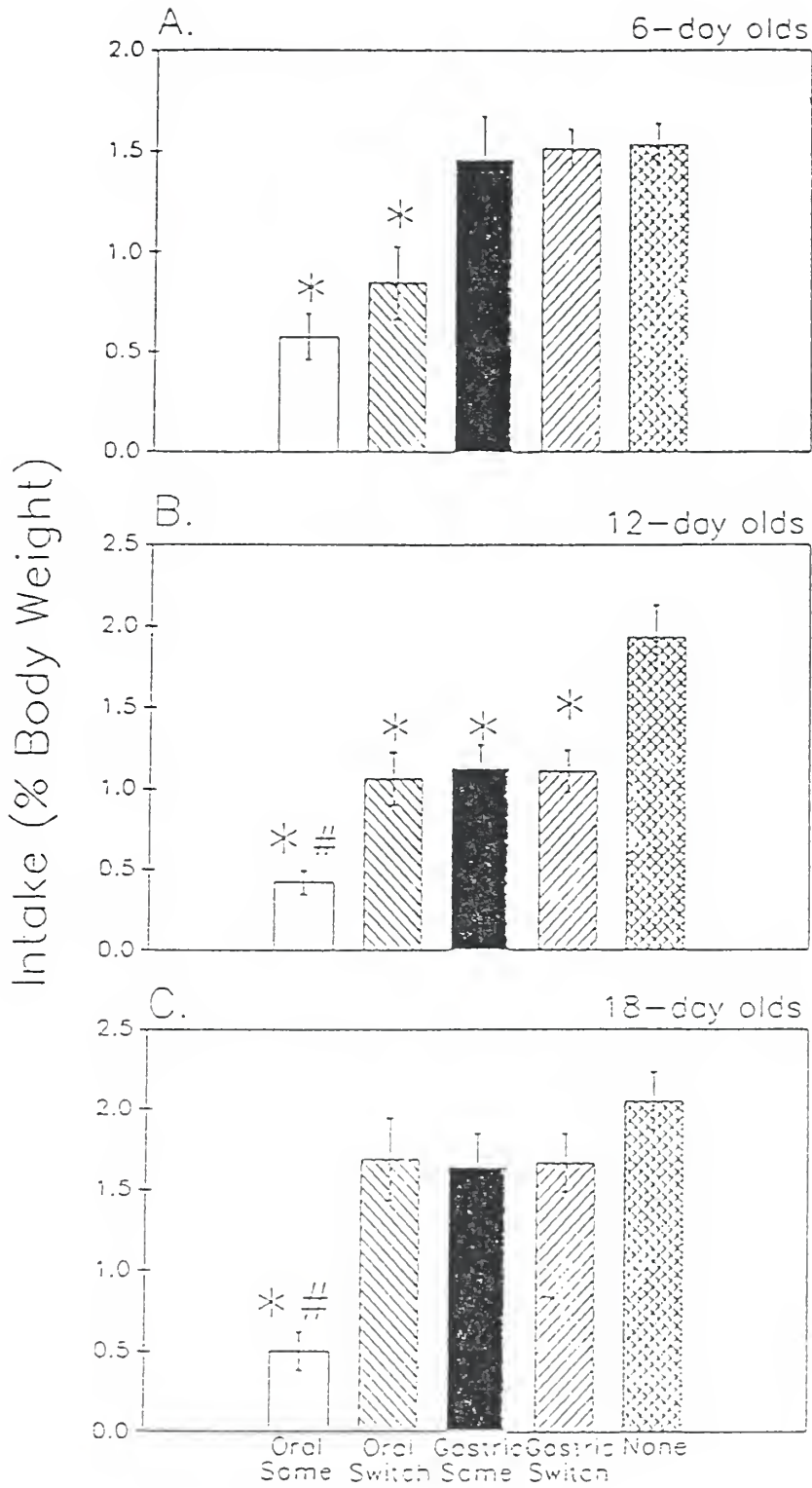


Figure 3.

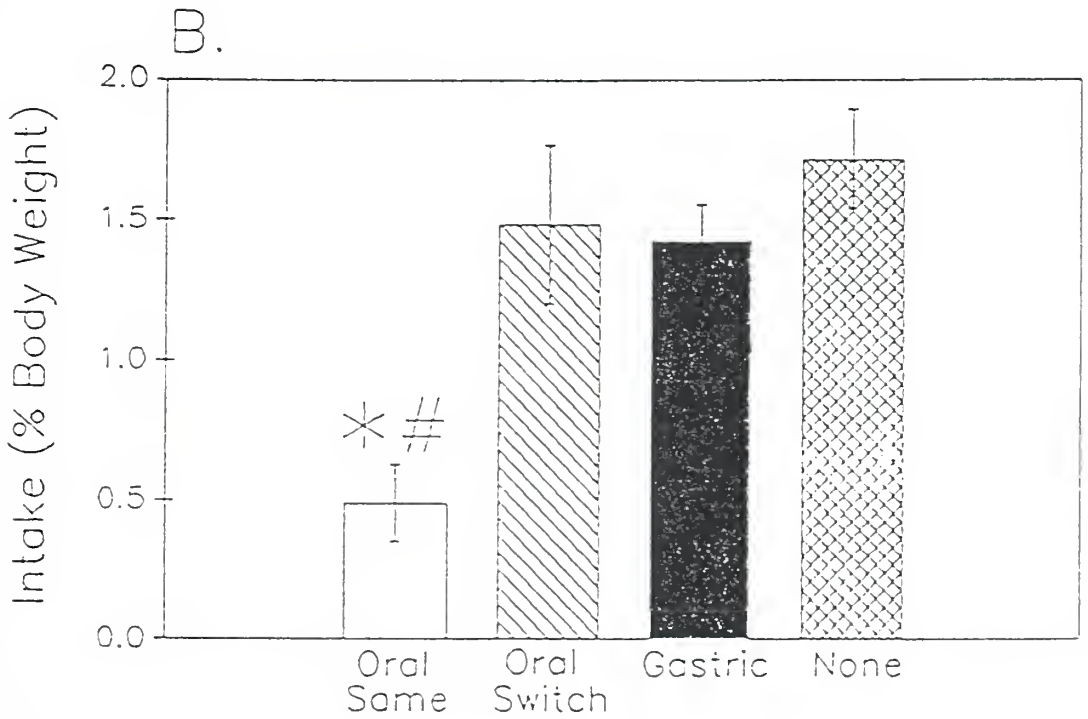
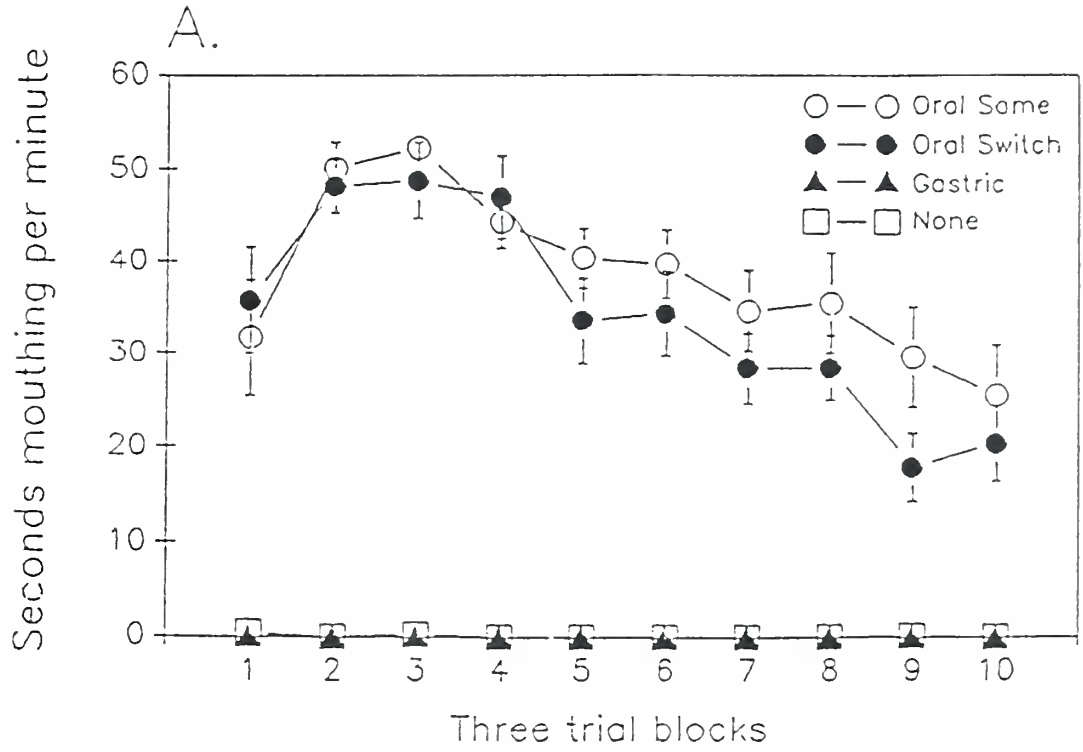


Figure 4.

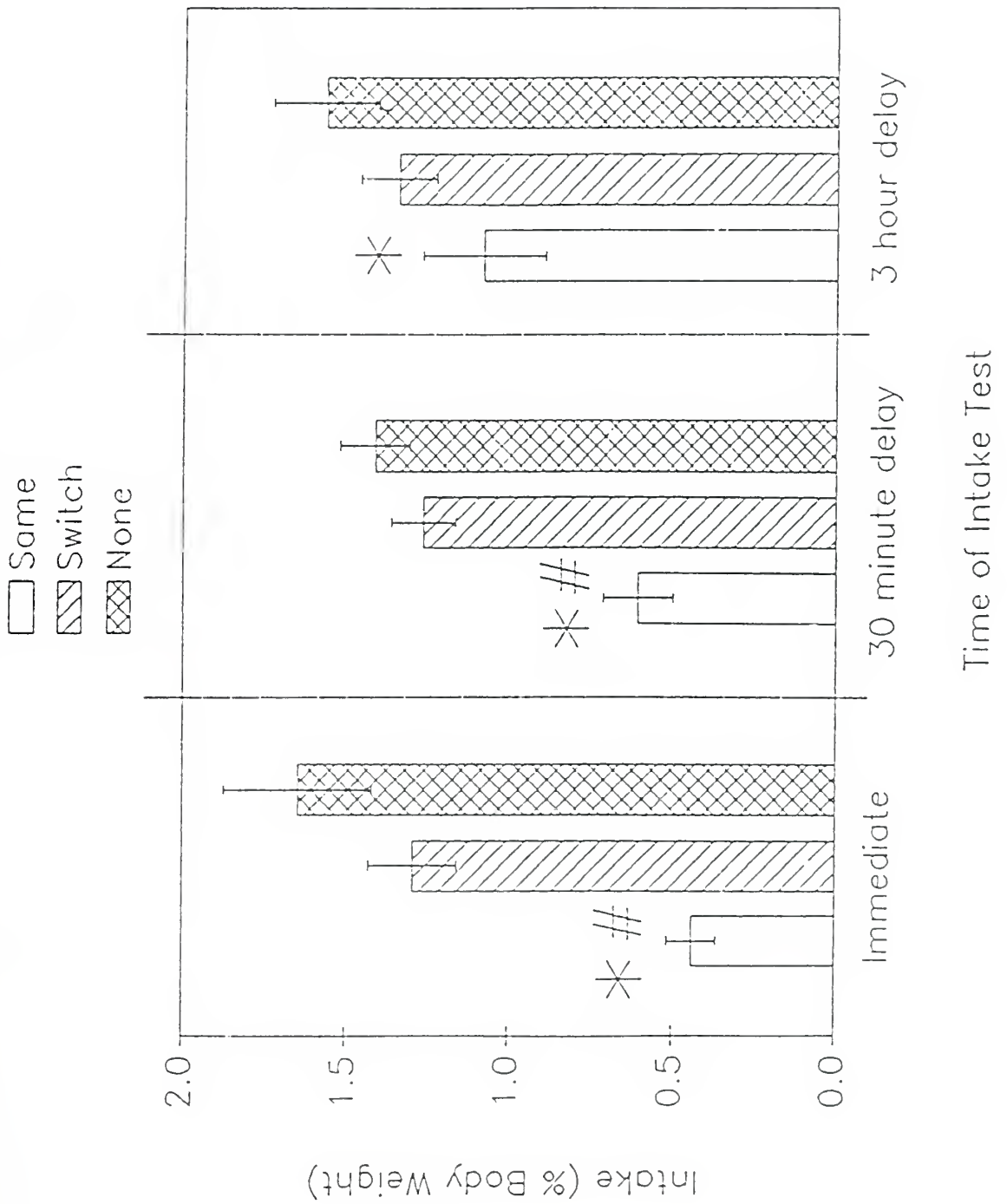
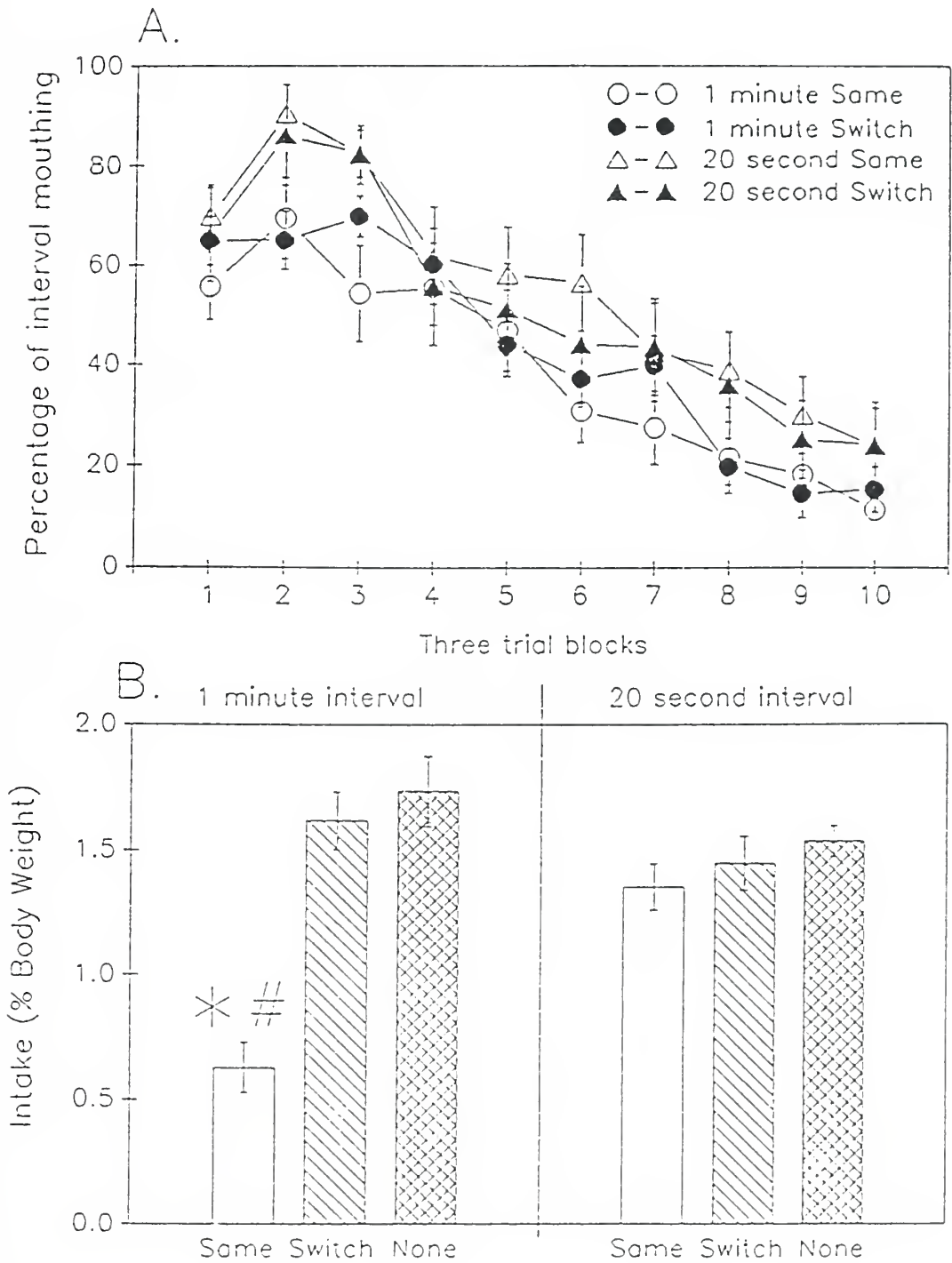


Figure 5.



CHAPTER III.

AN ELECTROMYOGRAPHIC ANALYSIS OF ORAL HABITUATION IN RAT PUPS

ABSTRACT

A rat pup shows decreases in mouthing activity in response to a series of repeated oral infusions of a diet, a change that is readily demonstrated with simple behavioral observations. The present study examined this change in oral responding using electromyographic (EMG) recordings to describe changes in the motor pattern during oral habituation. The decrease in duration of mouthing activity observed behaviorally was confirmed by determinations of mouthing duration from EMG records from the superficial masseter, anterior digastric, sternohyoideus and genioglossus muscles. Other profound changes occurred in the pattern of mouthing activity that were unappreciated with behavioral observations alone. The results from this experiment demonstrate that the cycle frequency, duration of activity and relative onset time of the mouthing motor pattern changes dramatically within an infusion. Different changes in the motor patterns were seen across habituation. Further, changes in the activity of one muscle (e.g. the genioglossus) can be relatively independent of changes in activity in other muscles (e.g. masseter and digastric). Thus, oral habituation has selective effects on different muscle involved in the mouthing motor pattern.

INTRODUCTION

Rat pups respond to the oral experience of a series of brief intra-oral infusions with steady decreases in levels of oral responsivity (Swithers-Mulvey, Miller & Hall, 1991). In previous studies, we have documented the similarity of this decline in mouthing responses to decrements seen in other response systems to repeated stimulus presentations, decrements termed response habituation; accordingly we have used the term oral habituation to describe the observed decreases in oral responsivity. A number of observations suggest that oral habituation may constitute a fundamental ingestive control that universally terminates ingestive episodes and that integrates other ingestion-related feedback signals. First, the pattern of oral habituation is affected by physiological signals; deprivation can increase levels of oral responding while gastric loads can decrease response levels (Swithers-Mulvey & Hall, 1991b; Swithers-Mulvey, Miller & Hall, 1991). Oral habituation experience also has strong effects on the amount of diet subsequently ingested; pups consume less of a diet to which they have habituated (Swithers-Mulvey & Hall, 1991a). Thus, oral habituation is a potent, diet-specific suppressant of ingestion. Further, these suppressive effects on intake are relatively long lasting; compared to pups that receive no infusions of diet, habituated pups continued to consume less of a diet even 3 hours after habituation experience. Finally, oral habituation and the integration of oral experience with gastric load signals, though not deprivation, are represented at a hindbrain level. Decerebrate pups continue to demonstrate oral habituation, and show more rapid habituation when gastric load signals are added to oral stimulation (Swithers-Mulvey, Mishu & Hall, 1991; Swithers-Mulvey & Hall, 1991b). Taken together, these results suggest that the oral habituation system in pups may represent a primary control of ingestion.

To this point, our behavioral characterization of oral responsiveness and habituation has largely relied on observations of mouthing responses to oral infusions. However,

behavioral observations provided only limited information on the specific organization of oromotor responding, a complex behavioral product involving many muscles. Additional information relevant to the production of oral behavior and modulatory influences on it can more directly gathered and studied by recording electromyographic (EMG) signals from muscles active in the opening and closing of the jaws, and in the movement of the tongue. The EMG technique has already proven useful in a quantitative analysis of the development of rodent oromotor activity related to suckling and chewing behaviors (Westneat & Hall, 1991). In these previous experiments, EMG signals from the superficial masseter, anterior digastric, sternohyoideus and genioglossus muscles were recorded from pups of several ages spanning the preweaning period while pups were either suckling from an anesthetized dam or engaged in chewing rat chow between the molar teeth. Results from this study demonstrated that while motor patterns for rhythmic suckling were quite different from chewing motor patterns, close similarities are seen between motor patterns for nipple attachment and molar chewing. These results suggest that study of EMG signals during ingestive behavior can provide significant new insight into aspects of behavioral control.

Thus, in the present experiment, EMG records of oral activity from four cranial muscles in 12-day-old rat pups were collected at beginning of the infusion series and compared to those after oral habituation had developed. The muscles examined were the superficial masseter, active during jaw closing (e.g. Hiilemae, 1971); the anterior digastric, active during jaw opening; sternohyoideus, anchoring the hyoid to the ventral skeleton and functioning in jaw opening (Crompton, Thexton, Parker & Hiilemae, 1977); and the genioglossus; the primary tongue protruder also involved in food manipulation (Herring & Scapino, 1973). During oral habituation, a brief (3 sec) infusion of sucrose solution was delivered into the front of the pup's mouth once every minute through an indwelling cannula implanted sublingually. The placement of the cannula allowed the pup to freely ingest or

reject the infused solution. Previous behavioral observations had indicated that following one 3 second such infusion, pups can display mouthing activity for 40-50 seconds prior to the delivery of the next infusion, considerably longer than may be necessary to transport the infused solution to the back of the mouth and swallow it. Because in this paradigm pups display mouthing activity both during active transport and well beyond the time necessary to swallow the infused solution, patterns of muscle activity could be examined both during active transport and during mouthing was likely unaccompanied by transport of the infusate. Recordings from the muscles during oral habituation were inspected for changes in motor patterns both within trials and across habituation. In addition, EMG records correlated with observational records permitted an independent confirmation of the precision with which mouthing behavior can be assessed from simple behavioral observations.

METHODS

Subjects

Subjects were 12-day old progeny of Charles River CD strain rats mated in our laboratory. Pregnant females were housed individually in laboratory. After they were bred, pregnant females were housed individually in polyethylene cages during the week prior to parturition and remained with their litters until the time of testing. Cages were checked for births daily between 0800 and 1700 hrs and pups found before 1700 hrs were considered 0 days of age. Litters were culled to 10 pups (5 male and 5 female) at 1 day of age. Water and Purina Formulab Chow (no. 5008) were available ad lib in the cages. The colony room was maintained at 21-23° C (40-70% relative humidity) on a 14:10 light-dark cycle.

Electrode and Cannula Placement

Bipolar, fine wire electrodes were constructed from 0.05 mm diameter, insulated,

stainless steel wires. Electrodes were threaded through a 27 gauge needle for implantation into muscles. Pups were anesthetized with methoxyflurane and a midventral incision was made posterior to the mandibular symphysis and anterior to the sternum. This incision exposed the sternohyoideus, superficial masseter, and anterior digastric muscles. Electrodes were implanted into the genioglossus by inserting the electrode tip through the digastric and geniohyoideus muscles and into the body of the genioglossus. Following implantation, electrodes were run subcutaneously to an incision on the dorsal surface of the body, above the cervical vertebrae. Electrode wires were then glued together to form a single cable that extended 40-50 cm from the pup. Wounds were closed with sutures (6-0 silk) and veterinary adhesive (Nexaband). Anterior oral cannula were implanted at this time as previously described (Hall, 1979). Pups were then housed individually in plastic cages lined with absorbent bedding placed inside warm, humid incubators for 18 hours prior to testing. Immediately following testing, pups were sacrificed and placement of EMG electrodes was verified. Data from a total of 5 pups (each from a different litter) were used in the analysis.

Ingestive Testing

Immediately prior to testing, pups were stimulated to urinate and defecate by stroking their perineal region with a soft brush. Pups were then placed into clear plastic test containers (6.5 x 11 x 12.5 cm) inside a warm, humid glass incubator. The oral cannula was attached to a length of plastic tubing which connected to a 5 cc syringe mounted in a Harvard infusion pump. A programmable timer (Chronotrol) controlled the action of the infusion pump. After a 15 minute accommodation period in the testing containers with the cannula leads attached, ingestive tests were begun. During testing, brief infusions of a 10% sucrose solution were delivered through the cannulas and pups' mouthing behavior was

scored continuously. Mirrors behind and under the clear floor of the pups' test containers allowed for easy viewing of pups' oral activity. 'Mouthing' behavior was defined as opening or closing of the jaws and/or movement of the tongue and was recorded second by second.

Electromyographic signals were preamplified close to the source by Grass HIP5G high-impedance probes, and then amplified 5,000 - 10,000 times with Grass 7P5A amplifiers. The EMG signals were monitored during the experiments on a computer and recorded on a four channel FM tape recorder for later analysis.

EMG analysis

The analog tapes of electromyographic activity were played into a NB-MIO-16 analog-to-digital converter driven by LabVIEW 2 virtual instrument software (National Instruments Corp.). The sample rate used to examine an entire trial was 500 points per second per channel. The sample rate used to examine within-trial bouts was 5000 points per second per channel. The digital record was analyzed by filtering the data with a high-pass Butterworth filter set at a sample rate of 5000Hz and a cut-off frequency of 60Hz. Each channel was visually inspected to determine the remaining baseline noise level, and a cut-off value was chosen below which all values were set to zero. The onset and offset point of each muscle burst within an EMG record could thus be objectively identified.

For each pup, EMG data from three separate trials across the testing period were analyzed. The first trial analyzed was within the first seven, the second trial analyzed was taken from trials 13-18 and the final trial analyzed occurred from trials 25-30. The measurement of duration and relative onset time variables from an electromyogram are illustrated in Figure 1. Description of the motor pattern of mouthing behavior consisted of a cycle frequency measure and the following seven EMG variables:

- 1) Masseter duration

- 2) Digastric duration
- 3) Sternohyoideus duration
- 4) Genioglossus duration
- 5) Onset time of digastric relative to onset of masseter
- 6) Onset time of sternohyoideus relative to onset of masseter
- 7) Onset time of genioglossus relative to onset of masseter.

For each of the three trials analyzed, EMG variables and cycle frequency were determined during a 3-5 sec sample taken from the period immediately following the oral infusion, within 20 seconds of the beginning of the trial. A second 3-5 sec sample period from each trial was taken from the period within 20 seconds of the end of the trial. For each sample, measurement of three separate bouts were made, and an average duration or offset score was computed from these three bouts and used in the analysis. In addition, the EMG records from 9 entire minutes were digitized from each pup and the number of seconds during which rhythmic activity occurred was determined.

Statistical analysis

A one-way, repeated measures ANOVA was performed on the mouthing observation scores (PC-SAS, release 6.03, Statistical Analysis Systems, Cary, NC). For the EMG data, separate two-way (Time Across Habituation X Time Within Trial) ANOVAs were performed on the duration, relative onset, and frequency measures. A coefficient of correlation was determined for the duration of mouthing observed behaviorally and the duration of mouthing determined from EMG records.

Results

The mouthing activity of these pups showed robust habituation over the course of the 30 trials ($F[29, 116] = 1.96, p < 0.01$; Figure 2). The levels of oral responding were somewhat higher overall than typically observed in our lab in unoperated pups. This finding suggests that while the surgery required to implant the EMG electrodes did not completely disrupt the behavior, pups may have been somewhat irritated by the presence of the electrodes or the cable leading from their dorsal surface, leading to increased arousal and mouthing. Mouthing activity as recorded from behavioral observations was closely correlated with mouthing activity as determined by EMG ($r = .83$; Figure 3). Thus, simple observation of the occurrence of mouthing is a reliable measure of the behavior.

Typical muscle patterns of oral responding to infusions consisted of initial activation of masseter, followed by contemporary activation of digastric and genioglossus and finally activation of sternohyoideus (Figure 1.) Analysis of EMG variables and cycle frequency revealed a number of changes in the motor pattern within trials and across habituation series. First, within trials, regardless of when they occur during the habituation session, cycle frequency of EMG activity was higher at the start of the trial than at the end of the trial (frequency at start of trial = 3.5 cycles/sec; frequency at end of trial = 2.4 cycles/sec; $F[1, 26] = 26.22, p < 0.001$; Figures 4 and 5A). The change in cycle frequency within trials was accompanied by a significant change in the duration of activity (overall duration at start of trial = 497 ± 14 msec; overall duration at end of trial = 611 ± 23 msec; $F[1, 95] = 18.82, p < 0.001$). A change in relative onset times was also observed with trials (start of trial = 650 ± 51 msec; end of trial = 1019 ± 109 msec; $F[1, 58] = 16.37, p < 0.001$; Figure 6).

Over the course of trials, cycle frequency also decreased as habituation developed, (frequency at beginning of habituation = 3.25 cycles/sec; middle of habituation = 3.05 cycles/sec; late in habituation = 2.55 cycles/sec; $F[2, 26] = 3.76, p < 0.05$; Figures 4 and 5). This decrease in cycle frequency as habituation progressed was accompanied by a

change in the relative onset time (onset at beginning of habituation = 72 ± 7 msec; middle of habituation = 79 ± 8 msec; late in habituation = 101 ± 19 msec; $F [2, 58] = 4.17$, $p < 0.05$). However, in this case, duration of activity was similar across habituation (beginning = 57 ± 2 msec; middle = 53 ± 2 msec; late = 54 ± 2 msec; $F [2, 95] = .83$, NS; Figure 6). In addition, at the end of trials after habituation had developed a qualitative change in the response appeared as EMG activity in genioglossus was absent in all 5 pups, and activity of the sternohyoideus was absent in 3 of 5 pups (Figure 7). The changes in activity of individual muscles across habituation and within trials are presented in Tables 1 and 2 and Figures 6 and 7.

Finally, although changes in amplitude of activity within muscles were not directly measured, visual inspection of the EMG records suggests that EMG amplitude remains relatively constant within trials, but may decrease with the onset of habituation, indicating that late in the habituation session fewer muscle fibers are recruited.

DISCUSSION

These results demonstrate that simple observation of mouthing activity is a reliable measure of the overall expression of the behavior. Observations of the duration of activity are closely correlated with durations of mouthing activity determined by EMG patterns.

In addition, these records of EMG activity provide a detailed description of the motor pattern during mouthing in four separate muscles related to ingestion. The motor pattern of mouthing in these 12-day-old pups is characterized by rhythmic activity in each of the four muscles measured. Within each cycle, only one burst of activity from each muscle is observed. Jaw closing indexed by superficial masseter activity alternates with jaw opening activity seen in the activity of the anterior digastric, with little overlap. Activity in the tongue protruder, the genioglossus during mouthing in 12-day olds is relatively synchronous with

digastric activity. Finally, during mouthing, activity of the sternohyoideus is considerably delayed relative to the digastric and genioglossus, with little or no overlap. While patterns of activity in adult animals during mouthing have not been recorded in these four muscles simultaneously, there is some evidence to suggest that the motor pattern of mouthing in pups is similar to the motor pattern in adults. In particular, the pattern of simultaneous activation of digastric and genioglossus muscles has been documented in adult animals mouthing in response to intraoral infusions of sucrose (Travers & Norgren, 1986).

Along with providing a detailed description of the basic motor pattern of mouthing, examination of EMG activity provides more detailed information about how mouthing behavior changes as a result of oral habituation. During oral habituation, behavioral observations indicate that the amount of time a animal spends mouthing decreases and EMG recordings indicate that when mouthing does occur, motor patterns change at various times within habituation testing. First, cycle frequency of mouthing decreases both within a trial and across habituation trials. The change in cycle frequency within trials is accompanied by changes both in the duration of muscle activity and the relative onset times of various muscles. The result of these changes is to make the cycle pattern appear more spread out at the end of a trial compared to the beginning. These changes in activity within a trial may be related to whether or not the animal is actively transporting the infused sucrose solution within the mouth. Early in the trial, when cycle frequency is highest, the infusate has just been delivered and the animal is manipulating it. By the end of the trial, the entire infusion has presumably been swallowed because the amount of diet infused within each trial was quite small (approximately 0.02 ml per trial). Changes in the motor pattern may reflect differences in muscle activity related to whether or not the animal is manipulating the infusate. On the other hand, the infusion of solution into the mouth likely triggers salivation and thus even at the end of a trial, the pup may still be manipulating

some solution. For this reason, the possibility must be considered that the change within trials reflects an alteration in some other property of the stimulus, such as a decrease in its perceived strength or acceptability.

Complementing these changes in activity within trials is an alteration of the motor pattern of mouthing across trials as habituation develops. Cycle frequency at the end of the habituation series is decreased compared to the frequency at the beginning of the series. However, the changes in the motor pattern accompanying this decrease across trials is different from the changes in the motor pattern seen within trials. Across trials, the duration of muscle activity remains similar, but the relative onset times are altered and the amplitude of EMG activity appears to diminish across habituation. Further, by the end of testing, the genioglossus muscle becomes inactive in all pups as does the sternohyoideus in 3 of the 5 pups while the masseter and digastric muscles continue to be rhythmically active in an alternating fashion. This dissociation of changes in activity of the genioglossus and sternohyoideus compared to the digastric and masseter may suggest a fundamental difference in function of the two sets of muscles. For instance, the action of the digastric and masseter may represent more reflexive responses to the presence of a solution in the mouth while the actions of the genioglossus and sternohyoideus may represent more appetitive responses to the quality of the stimulus. In this case, the effect of oral habituation on the mouthing pattern is to alter not only the likelihood that the behavior will occur at all but also the appetitive vigor of the responses that do occur.

In summary, EMG recordings from four muscles involved in mouthing activity show a close correlation to mouthing behavior recorded by observation, supporting the value of simple behavioral observations. These results further demonstrate that between the start of a trial and the end of a trial, the cycle frequency, duration of activity and relative onset time of activity change. Across habituation, cycle frequency, relative onset times and perhaps

amplitude change, but duration of activity remains similar. In addition, there is a substantial decrease or loss of activity in the genioglossus and sternohyoideus muscles across habituation. The differences in changes in the motor pattern within trials compared to across habituation suggest that separate influences may modulate mouthing behavior over the two time bases considered here.

TABLE 1.

Duration (Msec; Mean \pm SEM) of Activity in Individual Muscles
Within Trials and Across Habituation

=====						
Across Habituation						
Within Trial	Early		Middle		Late	
	Start	End	Start	End	Start	End

Masseter	40 \pm 5	57 \pm 9	43 \pm 4	55 \pm 10	43 \pm 3	62 \pm 7
Digastric	54 \pm 5	64 \pm 1	54 \pm 6	58 \pm 5	54 \pm 3	67 \pm 4
Sternohyoid	53 \pm 4	74 \pm 9	55 \pm 5	61 \pm 11	48 \pm 8	53 \pm 6
Genioglossus	56 \pm 4	61 \pm 6	47 \pm 4	55 \pm 10	53 \pm 9	*****
=====						

***** Genioglossus activity was absent in all 5 pups at this time period.

TABLE 2.

Relative Onset Time (Msec; Mean \pm SEM) of Activity in Individual Muscles
Within Trials and Across Habituation

=====						
Across Habituation						
Within Trial	Early		Middle		Late	
	Start	End	Start	End	Start	End

Digastric	32 \pm 4	62 \pm 4	50 \pm 3	60 \pm 10	50 \pm 3	158 \pm 45
Sternohyoid	92 \pm 12	136 \pm 9	105 \pm 13	132 \pm 16	100 \pm 13	264
Genioglossus	39 \pm 4	60 \pm 6	52 \pm 8	83 \pm 7	62 \pm 7	*****

***** Genioglossus activity was absent in all 5 pups at this time period.

FIGURE CAPTIONS

Figure 1. A sample electromyogram showing the measurement of duration of activity of the masseter and digastric muscle and the onset times relative to the masseter of the sternohyoideus and genioglossus muscles. The vertical line represents time zero, the start of the cycle.

Figure 2. Patterns of mouthing activity in 12-day old rat pups in response to a series of brief, intra-oral infusions of 10% sucrose. Each point represents the average number of seconds mouthing per minute averaged over blocks of 3 trials.

Figure 3. Relationship of number of seconds mouthing recorded behaviorally (Y-axis) and determined electromyographically (X-axis).

Figure 4. Cycle frequency of mouthing activity sampled at the beginning of trials and at the end of the trials across habituation.

Figure 5. Representative 1.5 second electromyogram of mouthing activity from one 12-day-old pup. A. Sample of mouthing activity at the start of a trial early in the habituation series. B. Sample of activity at the end of the same trial. C. Sample of mouthing activity at the start of a trial late in the habituation series, after habituation has developed. D. Sample of activity at the end of the trial shown in C.

Figure 6. Mean mouthing cycles of pups at various time points during habituation. Bar lengths represent the mean duration of activity in the muscle. Error bars to the right indicate SEM of duration for each muscle and error bars to the left indicate SEM of relative onset

time for that muscle. Activity cycles on the left indicate the start of a trial A) at the beginning of habituation; B) in the middle of habituation and; C) late in habituation. Cycles of activity on the right indicate the end of a trial D) at the beginning of habituation; E) in the middle of habituation series and; F) late in habituation. Note that in F, the actual relative onset time of the sternohyoideus muscle is approximately 260 msec, but has been displaced for illustrative purposes. Also in F, there is no indication of genioglossus relative onset time or duration because no activity was observed in the genioglossus muscle at this time in any pup.

Figure 7. Representative electromyograms from a pup at the beginning of habituation (left) and after habituation has developed (right). A). First 20 seconds of trial; B) Last 20 seconds of trial; C) First 20 seconds of trial; D) Last 20 seconds of trial. Note that in D both the sternohyoideus and genioglossus become inactive while the masseter and digastric remain active.

Figure 1.

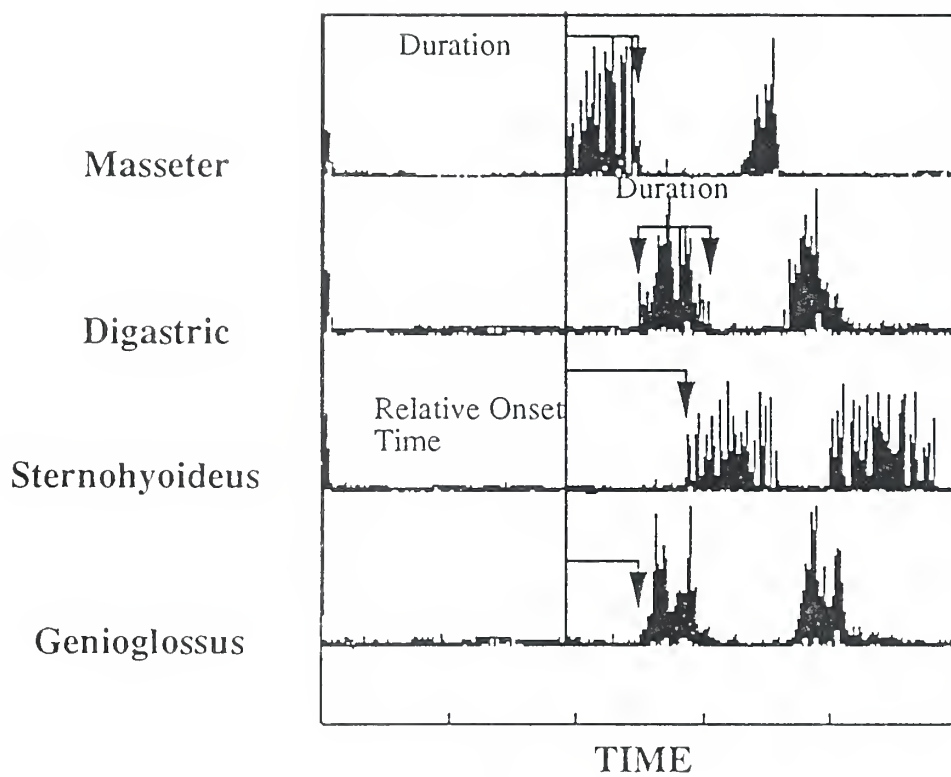


Figure 2.

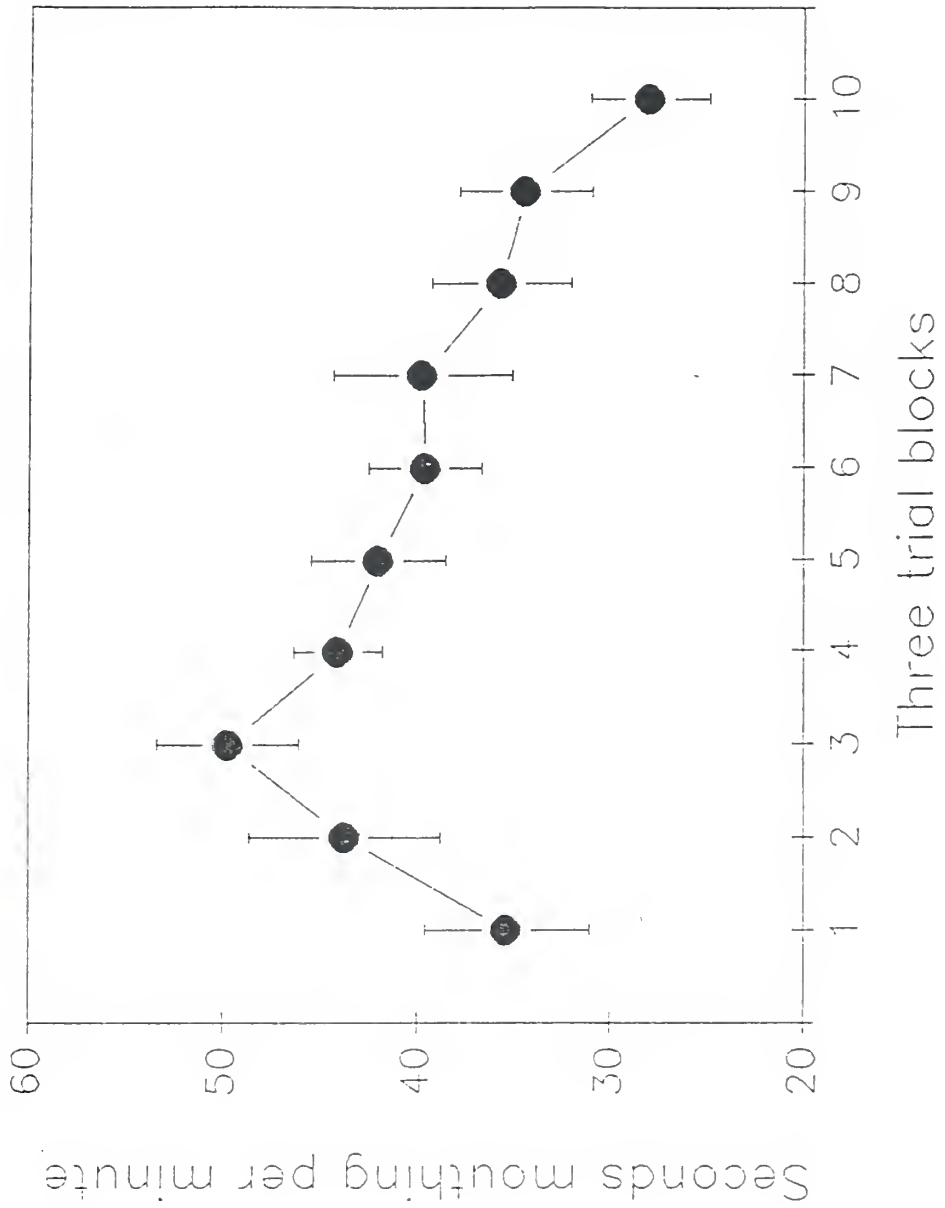


Figure 3

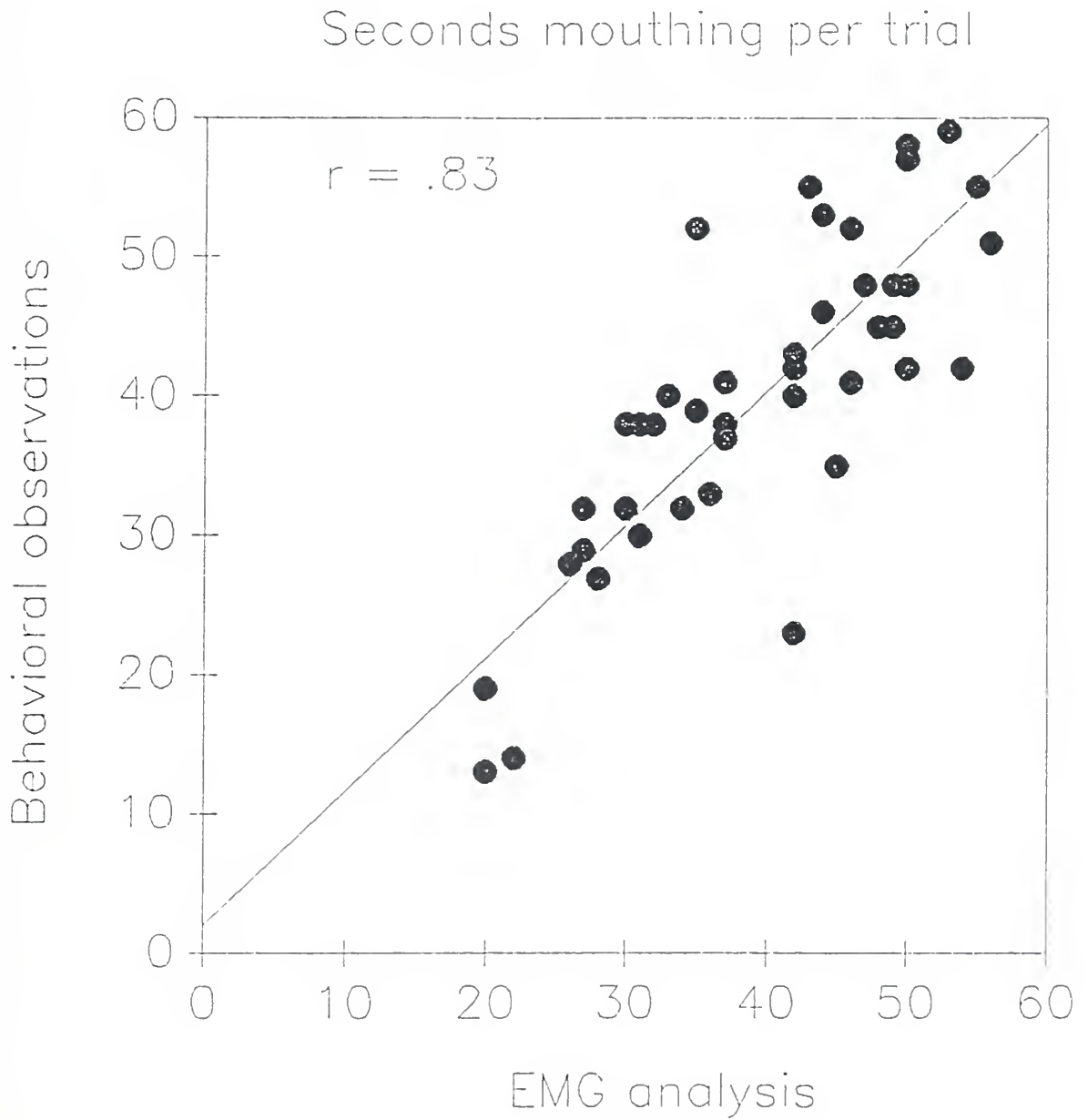


Figure 4.

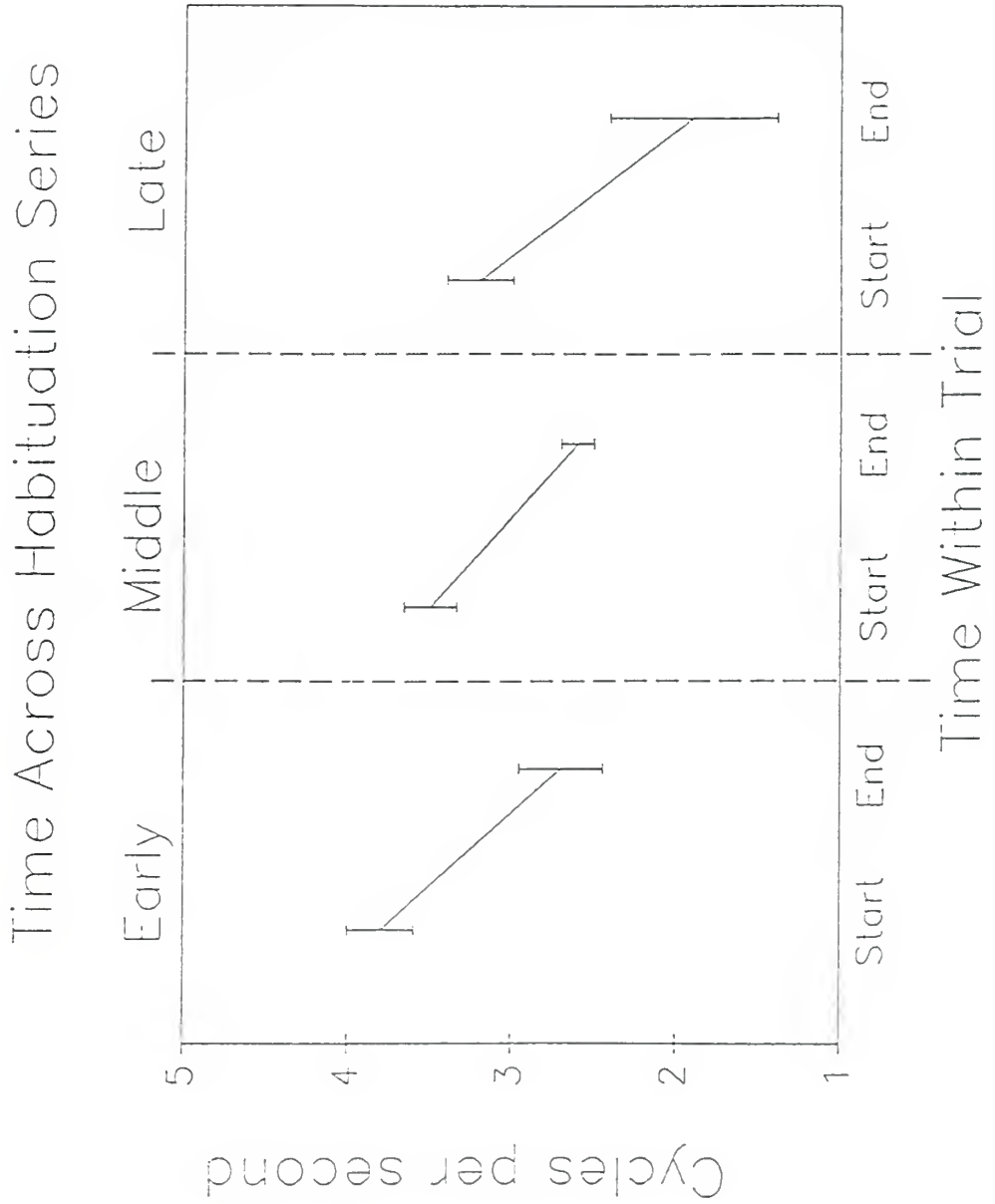


Figure 5.

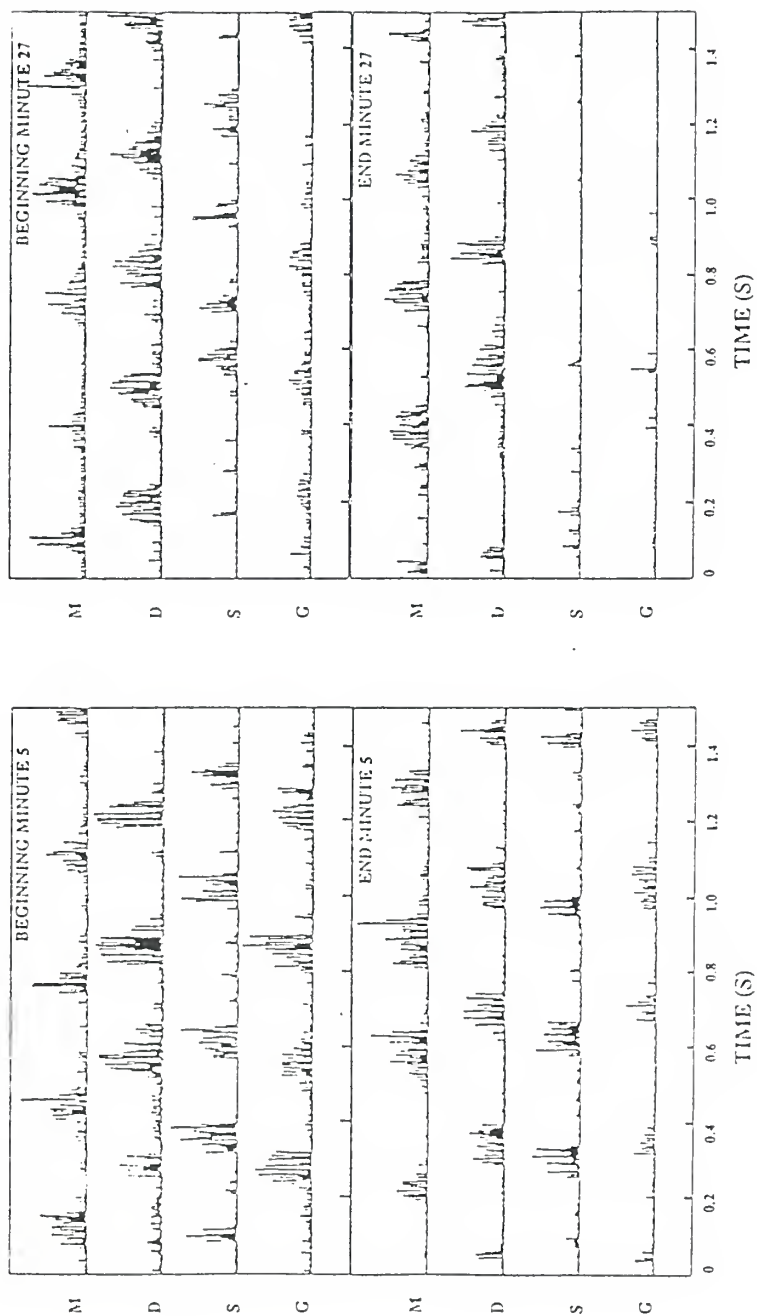


Figure 6.

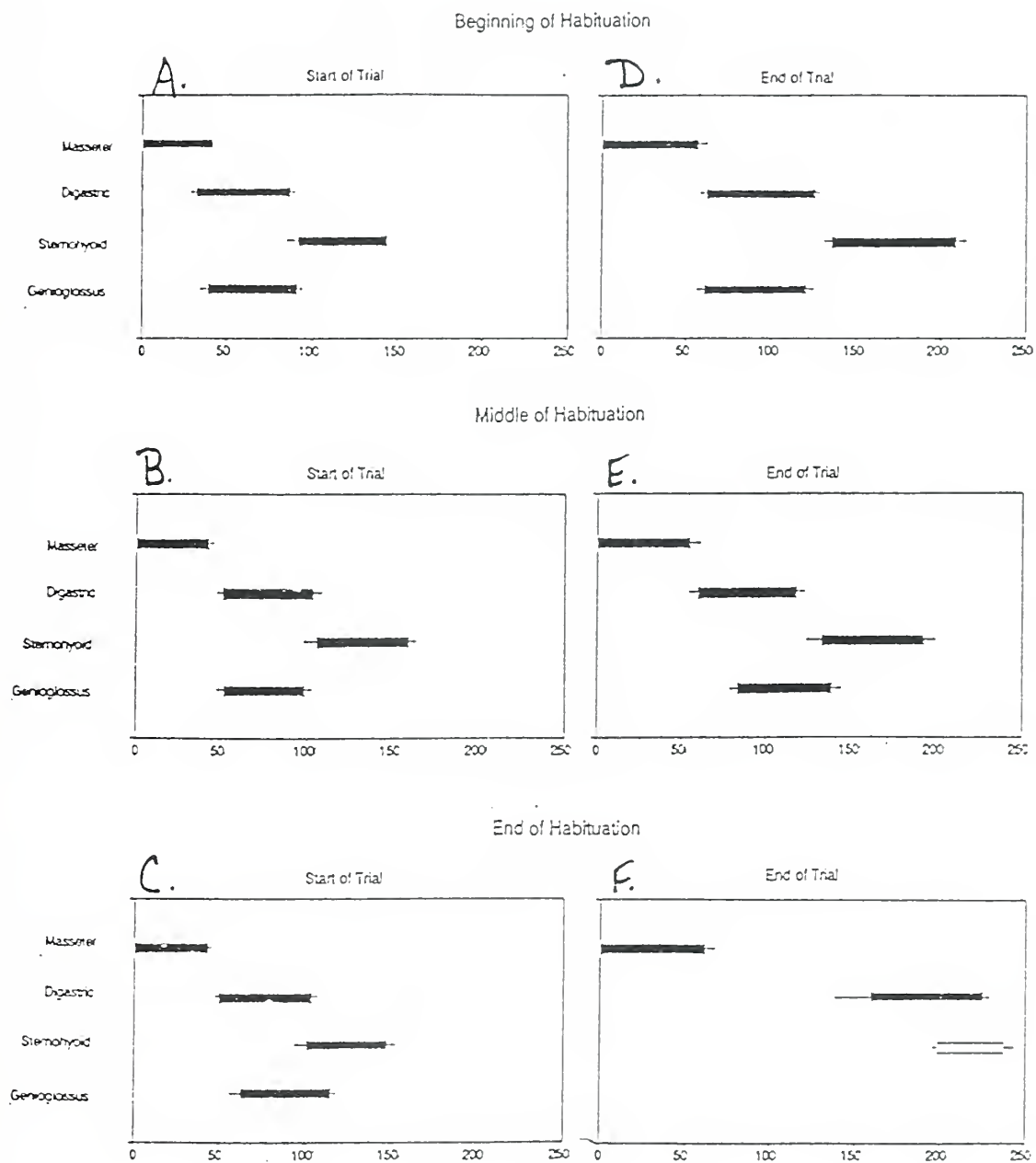
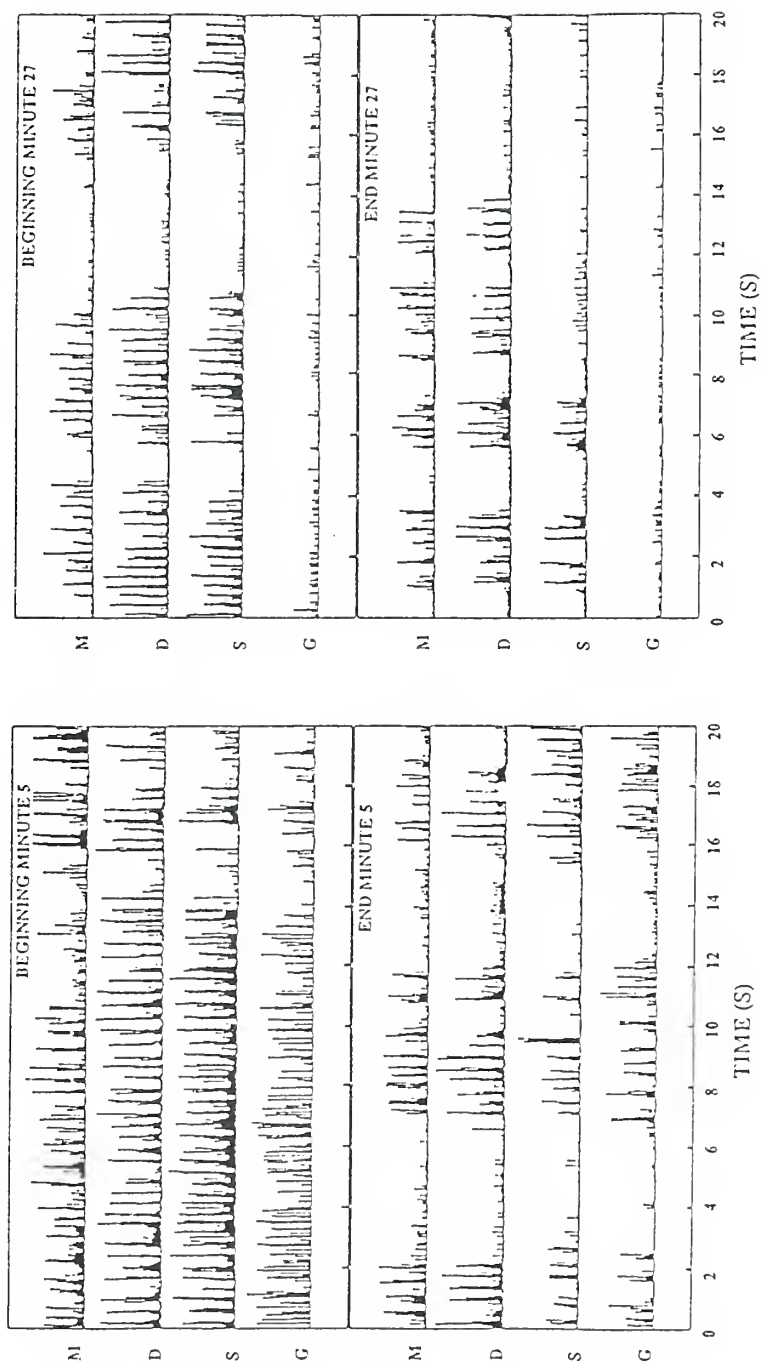


Figure 7.



CHAPTER IV.

ORAL HABITUATION IN RAT PUPS IS IN THE BRAINSTEM

ABSTRACT

We have previously demonstrated a decremental oral experienced-based control of ingestion in rat pups that is potent, diet-specific, and long lasting. This control of ingestion is revealed in the decreases in mouthing responses of rat pups to repeated oral stimulation, a phenomenon that is well described as 'oral habituation'. The present study examined the neural basis for oral habituation by examining the responses of decerebrate 10-day-old rat pups to repeated, brief infusions of a sucrose solution. Like neurologically intact pups, decerebrate pups showed distinct decreases in mouthing responses following a series of oral infusions. Thus, oral habituation is present in the hindbrain. However, although intact pups demonstrated sensitization by showing increased responding to the first few stimulus presentations, decerebrates failed to show a sensitization to the stimulation. These results suggest that while the brainstem alone is sufficient for the expression of oral habituation, the forebrain also influences oral responsivity in intact animals.

INTRODUCTION

The role of the mouth in and the necessity of oral experience for the production of satiety and intake termination have often been recognized (e.g. Kohn, 1951; Miller & Kessen, 1952; and more recently Mook, 1989; Rolls, 1981a). Prominent among the effects that oral experience can have, and one of the most direct contributions that sensorimotor experience in any domain can make, is the immediate diminution of the ongoing response based on recent stimulation or activation of the response. Stated in another manner, oral responses can habituate with use and the degree to which they habituate can influence their subsequent expression. Although the role of the mouth is not usually viewed by psychobiologists from a habituation perspective (though see Thorpe, 1966 and other ethological perspectives, e.g. Tinbergen, 1951), the concept of habituation provides a way of understanding what is meant by the "oral phase" or "oral factor" in feeding control, a control also frequently termed "oral metering".

We have previously shown that the responsiveness of the oral consummatory component in rat pups is dependent on its prior expression; in response to repeated brief intra-oral infusions of sucrose solution, oral activity declined (Swithers-Mulvey, Miller & Hall, 1991). That this decline was not due to post-ingestive consequences was demonstrated in the following ways: 1) by restricting the amount of diet infused to a small total volume; 2) by using sucrose, a sugar which young rats do not readily digest or absorb (e.g. Henning, 1987); and 3) by demonstrating that direct intragastric infusions alone failed to affect oral responsiveness. The decline in responsiveness was not due to fatigue because simply changing the flavor of the infused diet elicited an increase in mouthing response levels. In addition, oral experience with a diet suppressed the amount of a diet a pup subsequently consumed; this suppression of intake was evident even when tested 3 hrs after the oral experience (Swithers-Mulvey & Hall, 1991). In short, oral experience can significantly

modulate the subsequent expression of the consummatory component of ingestion. The decline in oral responsiveness to repeated stimulation resembles habituation as recognized and described in a number of other response systems, from response systems of invertebrates to response systems of humans. Thus oral habituation appears to be a strong potential modulator of the expression of the consummatory response and a potential control of ingestion in general.

The search for the neural representation of oral habituation might begin with the traditional view of brain mechanisms of feeding control, a view which has emphasized the influence of forebrain structures, in particular the hypothalamus. However, an alternative view attributes major integrative functions to lower structures in the brainstem (Grill & Kaplan, 1990). A considerable body of data from adult decerebrate animals demonstrates that, even in the absence of forebrain influences, decerebrates continue to demonstrate patterns of ingestive responding similar to normal animals and remain sensitive to a number of ingestion-related signals including sensory properties of the diet and some correlate of a gastric load (Grill, 1980; Grill & Norgren, 1978). Thus, the brainstem is a candidate site for production of oral habituation. The purpose of the present experiment was to assess the oral habituation capacities of the brainstem by transecting the brains of rat pups at a midbrain level and examining their responses to a series of oral infusions of sucrose following a 24 hr recovery (and deprivation) period.

METHOD

Subjects

Subjects were offspring of primiparous and multiparous Charles River CD strain rats maintained in the breeding colony in our laboratory. After mating, pregnant females were housed individually in polyethylene cages during the week prior to parturition. Cages were

checked for births daily between 0800 and 1700 hrs and pups found before 1700 hrs were considered 0 days of age. Litters were culled to 10 pups (5 male and 5 female when possible) at 1 day of age. The colony room was maintained at 21-23 °C (40-70% relative humidity) on a 14:10 light:dark cycle.

Surgery and cannula placement

When pups were 9 days of age, 22 to 24 hrs prior to testing, each pup was removed from its mother's cage and anesthetized with methoxyflurane (Metofane, Pitman-Moore) and cooling (Phifer & Terry, 1986). A small skin incision along the midline of the skull was made to expose lambda. Using a 23 gauge needle, a single transverse opening was made in the skull 1 cm anterior to lambda and 2 cm off the midline. The transverse opening extended approximately 4 cm. A rounded microknife was inserted into the opening, perpendicular to the surface of the skull and dropped to the floor of the skull. The knife was swung laterally in an arc in one direction, returned to midline, swung laterally in the opposite direction, then removed. The wound was closed with veterinary adhesive (Nexaband). In control pups, the same procedure was followed, but the knife was not inserted.

At the time of transection surgery an anterior oral cannula was also placed as previously described (Hall, 1979). Briefly, oral cannulas were constructed from a 10 cm length of polyethylene tubing (PE-10), flanged on one end and fitted to one end of a curved piece of stainless steel wire. Cannulas were then implanted by inserting the wire through the soft floor of the oral cavity just posterior to the lower incisors. The tubing was drawn down through the lower jaw until the flanged end was seated against the inner surface of the mouth. This placement permitted the pup to freely ingest or reject infused diets. Following surgery and cannula placement, all pups were housed in plastic containers lined with absorbent bedding inside warm (32-34 °C), humid (40-70% relative humidity) incubators until the time of testing. Two pups (one male, one female) from each of 6 litters were tested

in each condition.

Ingestive Testing

Immediately prior to testing, pups were stimulated to urinate and defecate by stroking their anogenital region with a soft brush. Pups were then weighed to the nearest 0.01 g and placed into clear plastic test containers (6.5 x 11 x 12.5 cm) inside a warm, humid glass incubator. The oral cannulas were attached to lengths of plastic tubing which were connected to 5cc syringes mounted in a Harvard infusion pump. A programmable timer controlled the action of the infusion pump. Pups were allowed a 15 minute accommodation period in the testing containers, with the cannula leads attached, prior to the habituation experience. During habituation experience, brief (3 sec) infusions of a 10% sucrose (w/v) solution were delivered once every minute for 30 minutes through the oral cannulas; pups' mouthing behaviors were scored continuously. Mirrors behind and under the clear sides of the test containers allowed easy viewing of pups' mouthing activity. "Mouthing" activity was defined as opening or closing of the jaws and/or movement of the tongue and was recorded second by second.

Verification of the transection

Immediately following testing, pups were sacrificed with an overdose of CO₂ and their brains were rapidly removed and placed into a 10% formaldehyde solution until sectioned. Sixty-micron thick sagittal sections were cut on a cryostat, mounted onto subbed slides, and were then stained with thionin. Stained sections from each pup were chosen at 4 levels (\pm 0.2mm and \pm 2.0mm) and projected onto drawings of parasagittal sections modified from an atlas of the 10-day-old rat brain (Sherwood & Timiras, 1970). The location of the cut was traced onto the atlas drawings. The lateral extent of the cut was determined by inspecting the farthest lateral sections in which the cut was still visible. Only pups with complete transections were included in the analysis.

Statistical analysis

A two-way repeated measures ANOVA (Condition X Trials) was performed on the duration of mouthing (PC-SAS, release 6.03, Statistical Analysis Systems, Cary, NC).

Tukey's Honestly Significant Difference Test was used for post-hoc comparisons between conditions, with a $p < 0.05$ taken as significant.

RESULTS

Decerebrations were considered complete in 9 pups. In each of these cases, the transection extended ventrally through the bottom of the brain and extended bilaterally into overlying cortex. Thus, the data from these 9 decerebrate and 11 control pups were included in the analysis. In the decerebrates, the majority of the cuts extended from the superior colliculus and through the interpeduncular nucleus (Figure 1). Dorsally, the cuts ranged from just anterior to the superior colliculus to the middle of the rostral-caudal extent of the superior colliculus. Ventrally, the most anterior cut was just anterior to the interpeduncular nucleus while the most posterior cut bisected the nucleus tegmenti pontis.

In response to repeated oral stimulation, both the decerebrate and intact 10-day-old pups showed significant decreases in mouthing activity over the course of testing (Figure 1; Main effect of Trials, $F[9, 162] = 40.06$, $p < 0.0001$), similar to patterns of oral habituation previously described in intact 12-day-old pups. However, the response patterns of control and decerebrate pups were not identical (Main effect of Condition, $F[1, 18] = 47.42$, $p < 0.0001$); the responses of decerebrate pups were lower initially and did not increase as did the responses of controls during early diet presentations. Further, behavioral observation of the mouthing activity of both groups indicated that in addition to mouthing for shorter durations, decerebrate pups demonstrated mouthing responses that were less vigorous and of smaller amplitude than those of control pups.

DISCUSSION

Decerebrate rat pups, like control pups, demonstrate oromotor habituation to orally infused sucrose solutions. The response patterns of both groups are qualitatively similar with both groups showing decreases in mouthing activity during the course of testing. Thus, the brainstem alone appears adequate for the expression of an experience-based control of ingestive behavior and thus expresses at least a short-term memory of ingestive experience.

While these results demonstrate the role of the brainstem in oral habituation, they also indicate a modulatory influence in the forebrain. In particular, forebrain signals may be necessary for the expression of sensitization, a term used to describe an initial increase in responding to a series of stimuli which along with habituation represents a fundamental, though separate, property of many behavioral system. The sensitization process is consistent with and perhaps equivalent to the well known arousal and incentive effects of oral ingestive stimulation. The non-specific, arousing sensitization process and the specific, decrementing habituation process interact to produce the observed pattern of behaviors. In intact animals, the result of the interaction of these two opposing processes is seen in an initial increase in responding over the first several trials followed by a steady decline. In contrast, no initial increase in response level is seen in decerebrate animals, and perhaps as a result of the absence of the sensitization process, the response levels of decerebrates are lower in a third of the trials than the responses of normal pups. Thus while habituation appears to reside in brainstem structures, the sensitizing or arousing properties of oral stimulation may be dependent on forebrain influences.

These patterns of declining responsiveness in normal pups have previously been demonstrated to depend on oral stimulation (Swithers-Mulvey, Miller & Hall, 1991). Other influences such as gastric fill or fatigue were ruled out by demonstrating that the decreases in responsiveness were specific to the diet infused orally. Switching the infused diet to a

second flavor restored responsiveness. We have not yet demonstrated the oral specificity of the decrementing response in decerebrates because the flavors used to demonstrate restored responding in intact pups differed primarily in their olfactory quality; grape versus cherry Kool-Aid. Because decerebration eliminates input from olfactory structures, our standard test of specificity will not work in decerebrate pups. Thus, while the pattern of responding in decerebrates resembles the oral habituation of intact pups, we cannot explicitly rule out other possible explanations, such as fatigue, for the decrement in responding of decerebrate pups. However, the most parsimonious explanation remains that the decline in responding seen in decerebrate pups is identical to the habituation-like decline in response of intact pups.

Such a habituation process could serve to integrate other ingestion-relevant signals about physiological state (i.e. those related to hydrational state, nutritional state, gastric fill, blood glucose levels, etc.) if features of habituation, such as rate of decrement in responsiveness, were modulated by these signals. Habituation could thus provide a way for an intrinsic, use-dependent process, to integrate its physiological state and recent feeding history and to express this integration in ongoing behavior by controlling oral responsiveness. We have previously demonstrated in intact pups that parameters of the oral habituation can be modulated by at least one physiological signal - deprivation state (13). An intriguing question thus remains: does this integration of other ingestion-relevant signals also occur at a brainstem level?

One might argue that the low level of representation of this oral habituation in pups is a developmental specialization in the Jacksonian sense (Jackson, 1931-32) and that in older animals such a control of ingestion may be accomplished by higher neural structures. On the other hand, this oral experience-based control of ingestion in decerebrate pups is consistent with the ingestive capacities previously demonstrated in chronic decerebrate adult

rats. Thus, an equally possible explanation is that oral habituation processes continue to be neurally represented in the brainstem of adult animals. The locus of habituation in adults remains an interesting question, particularly to the degree that the habituation mechanism may serve to integrate other control signals.

In summary, these results demonstrate that even in the absence of forebrain influences, including hypothalamic input, decerebrate rat pups continue to show habituation-like decreases to repeated oral infusions of a sucrose solution. At the same time, these data suggest that some modulatory controls of ingestion arise outside the brainstem. The response patterns of decerebrate pups appear to lack the sensitizing or arousing influences of oral stimulation, influences which therefore may depend on intact connections with the forebrain. Since oral habituation has previously been demonstrated to be a potent, diet-specific and long lasting control of ingestive behavior in intact animals, the present demonstration that the brainstem alone is adequate for the expression of oral habituation suggests that it may be a fundamental process in the control of ingestive behavior.

FIGURE CAPTIONS

Figure 1. A composite midsagittal drawing showing the level of the decerebration. Adapted from Sherwood & Timiras with permission. Copyright (c) Regents of the University of California, 1970.

Figure 2. Patterns of mouthing responses of intact and decerebrate 12-day-old rat pups to brief oral infusions of 10% sucrose delivered once every minute. Each point represents the average (\pm SEM) number of seconds mouthing per minute over blocks of three trials.

* $p < 0.05$ compared to control (Tukey's HSD).

Figure 1.

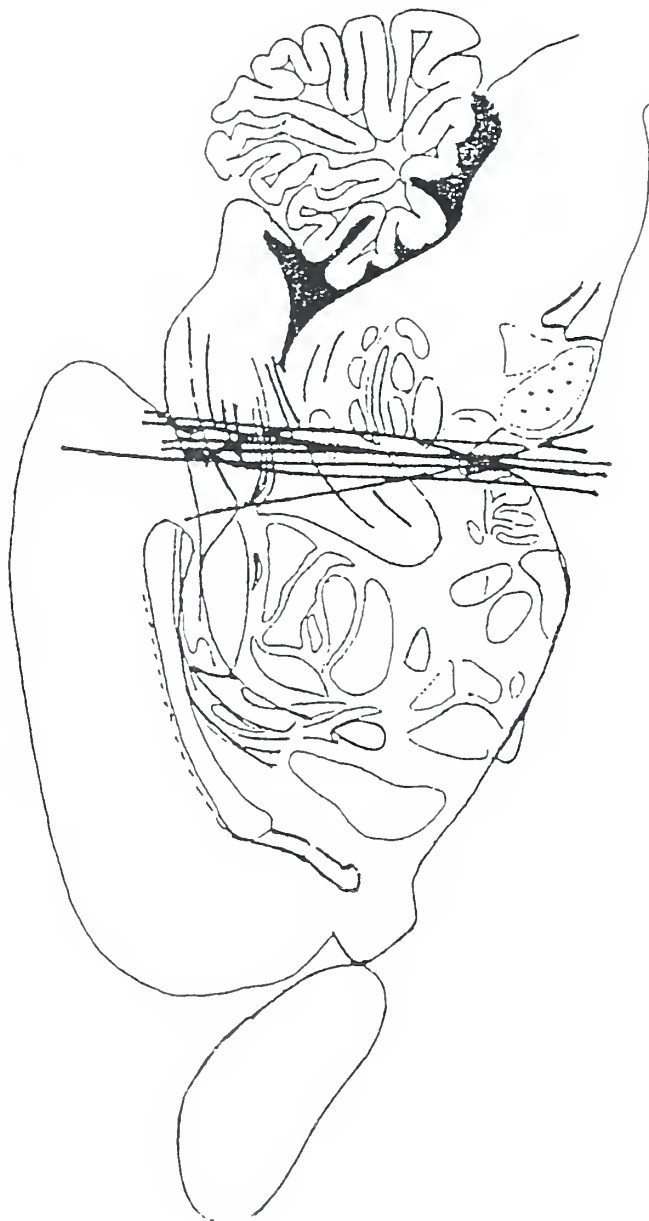
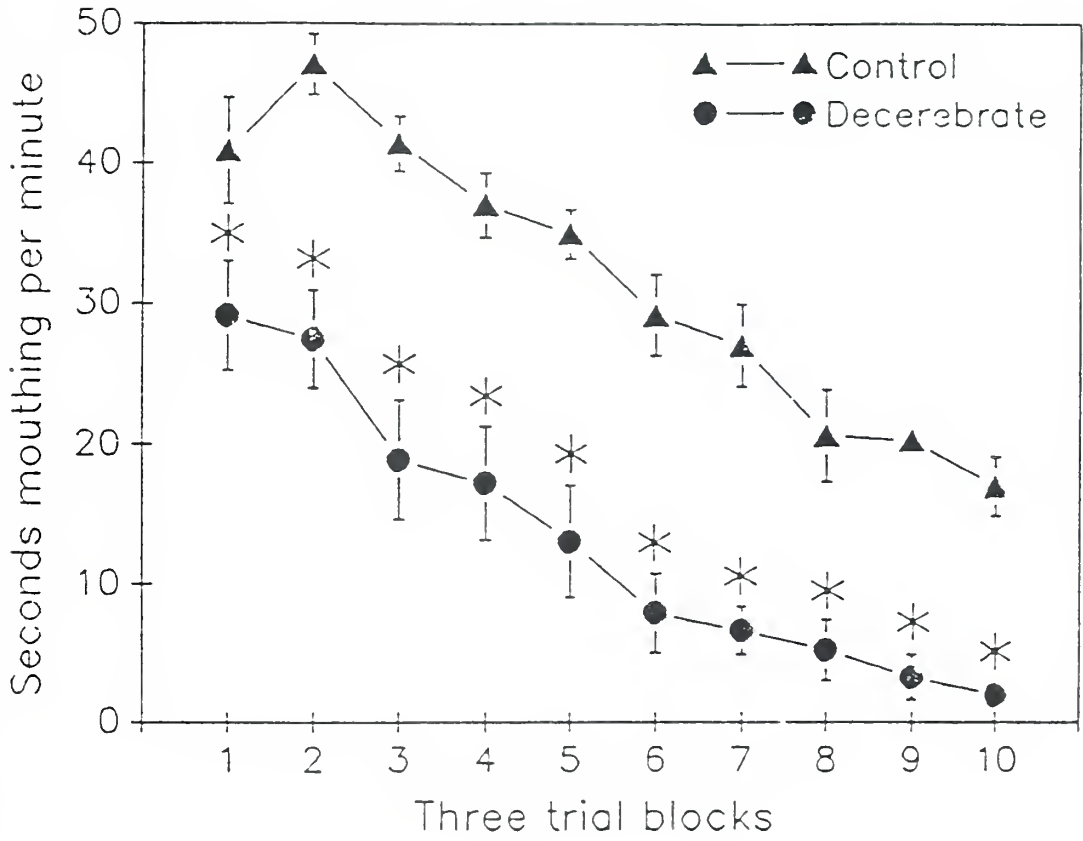


Figure 2.



CHAPTER V.

THE BRAINSTEM INTEGRATES ORAL HABITUATION AND GASTRIC FILL BUT NOT
DEPRIVATION IN RAT PUPS

ABSTRACT

Oral habituation has potent and long-lasting effects on intake in rat pups and may represent a fundamental control of ingestive behavior. Decerebrate pups demonstrate oral habituation and the integration of gastric signals, thus substrates for habituation are present in the hindbrain. But we show here that the forebrain is necessary for the deprivation-induced modulation of gastric load effects on oral habituation. Although the oral habituation system is capable of integrating many ingestion-related physiological signals, the integration of these signals appears to depend on separate mechanisms.

Studies of ingestive behavior in developing rat pups have suggested that an oral, experience-integrating mechanism may constitute a fundamental control of ingestive episodes. In these studies, young rat pups received a series of brief intra-oral infusions of a sucrose or saccharin solution through fine indwelling cannula. Examination of patterns of oral activity of rat pups to brief, repeated oral infusions demonstrates that oral responsiveness decreases dramatically during a series of infusions and that this decrement in mouthing responsiveness is not dependent on postingestive consequences or fatigue (Swithers-Mulvey, Miller & Hall, 1991). The decrement in oral responsiveness to repeated stimulus presentations resembles response habituation as described in a number of other response systems in rat pups (e.g. Peeke & Petrinovich, 1984; Thompson & Glanzman, 1976) as well as in animals in general (Lorenz, 1950; Tinbergen, 1951). A further parallel between the decremental oral process described here and habituation in other systems is the coinciding expression of sensitization processes; oral responsiveness often increases following the first several infusions before decrementing.

Oral habituation has been demonstrated to be a potent, diet-specific and long-lasting control of ingestion in rat pups (Swithers-Mulvey & Hall, 1991a). Pups that have habituated to oral infusions of a flavored solution subsequently consume less of that solution in an ingestive test. This suppression of intake is diet-specific in pups 12 days of age and older. Diet-specific suppression of intake is evident even when tested 30 minutes following habituation and oral habituation continues to suppress intake even after 3 hrs.

Recent work has demonstrated that oral habituation is neurally represented at a low level; habituation continues to be expressed in rat pups following decerebration (Swithers-Mulvey, Mishu & Hall, 1991). However, because decerebrate pups fail to demonstrate sensitization, some modulatory influences on oral habituation appear to require intact forebrain connections. This low-level neural representation of oral habituation, along with its

specific, potent and long-lasting effects on intake, suggests that it may be a fundamental control of ingestive behavior. Such a habituation process may serve to integrate other ingestion-relevant signals about physiological state (i.e. those related to hydrational state, nutritional state, gastric fill, blood glucose levels, etc.) by modulation of features of habituation (e.g rate of decrement in responsiveness or duration of effect). The present experiments were designed to examine the integrative capacities of the oral habituation system in response to a relatively simple signal - gastric fill. In addition, the neural level at which this integration was accomplished was investigated by examining the effects of a gastric load on patterns of habituation in decerebrate pups.

Pups were tested at 12 days of age¹ after 6 hrs deprivation to allow stomachs to empty. During habituation, control pups received a series of brief infusions delivered through indwelling oral cannulas². A second group of pups received these oral infusions as well as receiving a gastric load delivered through indwelling gastric cannulas beginning at

¹Subjects were progeny of Charles River CD strain rats maintained in the breeding colony in our laboratory. Cages were checked for births daily between 0800 and 1700 hrs and pups found before 1700 hrs were considered 0 days of age. Litters were culled to 10 pups (5 male and 5 female when possible) at 1 day of age. The colony room was maintained at 21-23 C (40-70% relative humidity) on a 14:10 light:dark cycle.

²Six hrs prior to testing, pups were removed from the mother's cage and oral and gastric cannulas were implanted under methoxyflurane anesthesia as previously described (Hall, 1979). Anterior oral cannulas were constructed from a 10 cm length of polyethylene tubing (PE-10), flanged on one end and implanted through the soft floor of the oral cavity just posterior to the lower incisors, with the flanged end seated against the inner surface of the mouth. This placement permitted the pup to freely ingest or reject infused diets. Gastric cannulas were constructed of a 30 cm length of PE-10 tubing and were positioned so that the flanged end rested against the inside surface of the stomach while the free end passed through the stomach and out the left abdominal wall. Following cannula placement, all pups were housed in plastic containers lined with absorbent bedding inside warm, humid incubators until the time of testing. Pups were placed into clear plastic test containers (6.5 x 11 x 12.5 cm) inside a warm, humid glass incubator. The oral and gastric cannulas were attached to lengths of plastic tubing which were connected to 5 cc syringes mounted in a Harvard infusion pump. A programmable timer controlled the action of the infusion pump. Pups were allowed a 15 min accommodation period in the testing containers, with the cannula leads attached, prior to the habituation experience. During habituation, small infusions of a 10% sucrose solution were delivered once every minute for 30 minutes through the oral cannula. A 2 ml gastric load of 10% sucrose was delivered to half of the pups continuously through an intragastric cannula over a 5 min period. Two pups from each of 6 litters were tested in each condition (n = 12).

the same time as the first oral infusion. Pups' mouthing behaviors were scored continuously during testing. "Mouthing" activity was defined as opening or closing of the jaws and/or movement of the tongue and was recorded second by second.

Oral activity in response to a series of intra-oral infusions of diet habituated. The addition of a gastric load to the oral stimulation resulted in similar responding of control and loaded groups to the first two oral infusions followed by a sharper decrease in responsiveness in the gastric load group by the third infusion (Fig. 1). Responses to all infusions following the third infusion were significantly lower in pups that received both oral and gastric stimulation³. These results indicate that, in mildly deprived pups, oral habituation can be significantly enhanced by the addition of a concurrent gastric load.

The next experiment examined the neural substrates mediating the effects of a gastric load which were demonstrated above. While traditional views of the neural control of ingestive behavior have attributed a large role to forebrain processes, an alternative view has emphasized the competence of the hindbrain with respect to many features or properties of ingestion (Grill & Kaplan, 1990). Adult, chronic decerebrate rats retain a number of ingestive capacities, among them full expression of taste preference and aversion as well as responsiveness to gastric loads (Grill, 1980; Grill & Norgren, 1978), and decerebrate pups remain capable of expressing oral habituation (Swithers-Mulvey, Mishu & Hall, 1991). Thus, it is possible that the effects of a gastric load on oral habituation are mediated at a brainstem level. We therefore examined whether the decrement in oral responding of decerebrate pups would be enhanced by the addition of a gastric load. Because pups are given 24 hrs post-surgical recovery time and hence 24 hrs deprivation, a surgical control group deprived for 24 hrs was used. This control group thus also allowed

³A two-way repeated measures ANOVA was performed and Tukey's Honestly Significant Difference (HSD) test was used for post-hoc comparisons between different conditions with a $p < 0.05$ taken as significant.

for an assessment of the effects of more lengthy deprivation on integration of gastric fill signals with oral habituation processes.

Transection surgery was performed on two groups of 11-day-old pups⁴, 24 hrs prior to testing (Fig. 2). Two further groups served as surgical controls. During habituation testing, one group of decerebrate pups and one control group received a series of oral infusions alone as described above, while the remaining decerebrate and control pups received a series of oral infusions and a gastric load identical to the one delivered in the first experiment.

While all groups demonstrated habituation of responses to the oral infusions, there were significant differences in the responses of pups in different conditions (Fig. 3). In decerebrate pups, addition of a gastric load resulted in more rapid decreases in mouthing responses starting with the fourth oral infusion. In contrast, and rather unexpectedly, in the 24-hr deprived neurologically intact group, oral habituation was unaffected by the addition of a gastric load. At no point during testing were the responses of intact pups without a gastric load significantly different from intact pups with a gastric load. These results are in sharp contrast to the clear effects of the same gastric load seen both in 24-hr deprived decerebrate pups (Fig. 3) and in 6-hr deprived neurologically intact pups (Fig. 1).

These data provide evidence that the pattern of decrementing oral responsiveness -

⁴Decerebrations were made by transection carried out under Metofane anesthesia. A longitudinal incision was used to expose the skull and a small hole was drilled with a 23 gauge needle just anterior to lambda and just off the midline. A rounded micro-knife was inserted through the drilled hole to the base of the skull, then moved through a lateral arc up each wall of the cranium. The incision was closed with veterinary adhesive. Surgical controls had the skull exposed and a hole drilled in the skull, but the knife was not inserted. Oral and gastric cannulas were also implanted in all pups at this time as described above. Pups were then placed in a warm, humid incubator for 24 hrs prior to testing. Verification of transections was accomplished by removing the brain after sacrifice with CO₂, fixing it, taking frozen sections at several levels into the sagittal plane, and staining with thionin. The level and extent of decerebration were documented by reconstruction on atlas drawings (Sherwood & Timiras, 1970). Transection surgery was performed on two pups from each of 6 litters. Decerebrations were judged complete in 9 decerebrate pups that received only oral stimulation and 8 decerebrate pups that received both oral stimulation and gastric loads. In each of the control groups, n's = 11.

oral habituation - may be modulated by a number of ingestion-related physiological signals. First, mildly deprived pups respond to a gastric load with more rapid decreases in oral responsiveness. This modulation of responsiveness occurs rapidly, with differences in mouthing behavior apparent following the third oral infusion. It is unclear what aspect of the gastric load is most relevant in modulating oral responsiveness, or whether the relevant signals arises from gastric or post-gastric effects of the load. Given the rapidity of the compensatory response, gastric or early post-gastric signals appear most likely. In other ingestive tests, pups at these ages are known to be responsive to gastric fill per se. In addition, pups are sensitive to osmotic properties of gastric loads, as well as being sensitive to some nutritive signals by this age (see Hall, 1990). It is, however, unlikely that in this experiment the effect of a gastric load is due to a caloric or metabolic signal because 12-day-old pups have very low levels of sucrase enzyme activity (e.g. Henning, 1987) and do not readily digest and absorb the sucrose solutions used in this test⁵.

The gastric-load induced decrease in oral responsiveness is itself subject to modulation in intact pups. In the present study, increasing the length of deprivation prior to testing resulted in a disregard for the gastric fill signal; patterns of habituation were similar whether or not the gastric load was delivered. This deprivation-induced modulation of the effects of a gastric load on oral habituation may result from a number of processes. Perhaps deprivation acts to directly decrease sensitivity to the volume of stomach fill. A related possibility is that sensitivity is not altered, but that pups deprived for 24 hr begin the tests with emptier stomachs than 6-hr deprived pups and since equivalent loads were given to both groups, the load failed to reach some 'threshold' for effect in the more deprived

⁵It is possible that the gastric infusion of a solution that is not easily digestible may suppress responsiveness by some non-specific illness producing mechanism. This appears unlikely, as in this experiment 24-hr deprived intact pups failed to respond to the gastric load, and as we have previously demonstrated that when ingestion is tested 2 hrs following a similar gastric load of sucrose in 9-day-old pups, intake is not suppressed (Swithers & Hall, 1989b).

pups. This seems unlikely however, because 6-hr deprived pups do show decreased responding well before the entire gastric load has been delivered and the total volume of gastric load delivered represents a relatively large proportion of the volume 24-hr deprived pups would voluntarily consume at this age (Hall & Bryan, 1980). An additional possibility is that deprivation may enhance sensitivity to the caloric or metabolic properties of gastric or post-gastric contents. If this were the case, the substances loaded in our experiment would be insufficient to alter oral activity because they had little caloric value. Further experiments using larger loads as well as loads which pups easily digest may help to distinguish among these possibilities.

Regardless of the nature of the gastric-load and deprivation related signals, results from decerebrate pups argue that signals related to deprivation and those mediating responsiveness to a gastric load are neurally separable. Decerebrate pups decrease their oral activity more quickly in response to a gastric load. Thus, the forebrain influences appear unnecessary for the integration of gastric load and oral experience-related signals. But the brainstem is insufficient for normal responsiveness to deprivation; if deprivation were mediated by the brainstem alone, then the 24-hr deprived decerebrate pups should have failed to incorporate the gastric load signal, like the intact 24-hr deprived pups. This separation of gastric and deprivation-related signals modulating oral responsivity parallels our previous findings on the neural separation of oral habituation from sensitization processes in decerebrate pups (Swihlers-Mulvey, Mishu & Hall, 1991).

Our findings of a brainstem representation of the integration of gastric load signals with oral habituation may be a consequence of studying these processes in developing animals. It is possible that as animals mature, these processes are re-represented at higher neural levels (Jackson, 1931-32) and brainstem control diminishes. But at least some of the capacities demonstrated here to depend on the brainstem alone in pups have also been

observed in chronic decerebrate adults. Namely, adult decerebrate rats remain both sensitive to the sensory properties of diets and responsive to some correlate of a gastric load (Grill, 1980; Grill & Norgren, 1978).

This oral habituation preparation in young rat pups provides the ability to isolate oral processes influencing ingestion and to anatomically identify neural substrates involved in their modulation. While the present experiments describe results of gross manipulations (i.e. large gastric loads and transections), the clear results of these manipulations suggest that with further study, the oral habituation procedure has the potential to yield important information about more subtle ingestive controls as well as more precise descriptions of how they are neurally mediated.

Figure Captions

Figure 1. Patterns of decremmenting mouthing activity (means \pm SEM) in 6-hr deprived pups in response to a series of oral infusions of 10% sucrose (Trials, $F[29, 638] = 30.20$, $p < 0.0001$). Beginning with the third infusion, the responses of gastric load pups were significantly lower than control pups (Condition, $F[1,22] = 84.56$, $p < 0.0001$; Trials X Condition, $F[29, 638] = 3.93$; $p < 0.0001$).

Figure 2. Mid-sagittal composite atlas (adapted from Sherwood & Timiras, 1970; Copyright (c) Regents of the University of California, 1970) drawing of level of transection in all decerebrate pups (control and gastric load).

Figure 3. Patterns of decremmenting mouthing activity (means \pm SEM) in 24-hr deprived intact and decerebrate pups (Trials, $F[29, 1015] = 30.16$, $p < 0.0001$). In neurologically intact pups, gastric loads had no effect on mouthing activity while in decerebrate pups, gastric loads reduced the level of mouthing activity beginning with the fourth trial (Condition, $F[3, 35] = 50.76$, $p < 0.0001$; Trials X Condition, $F[87, 1015] = 2.45$, $p < 0.0001$).

Figure 1.

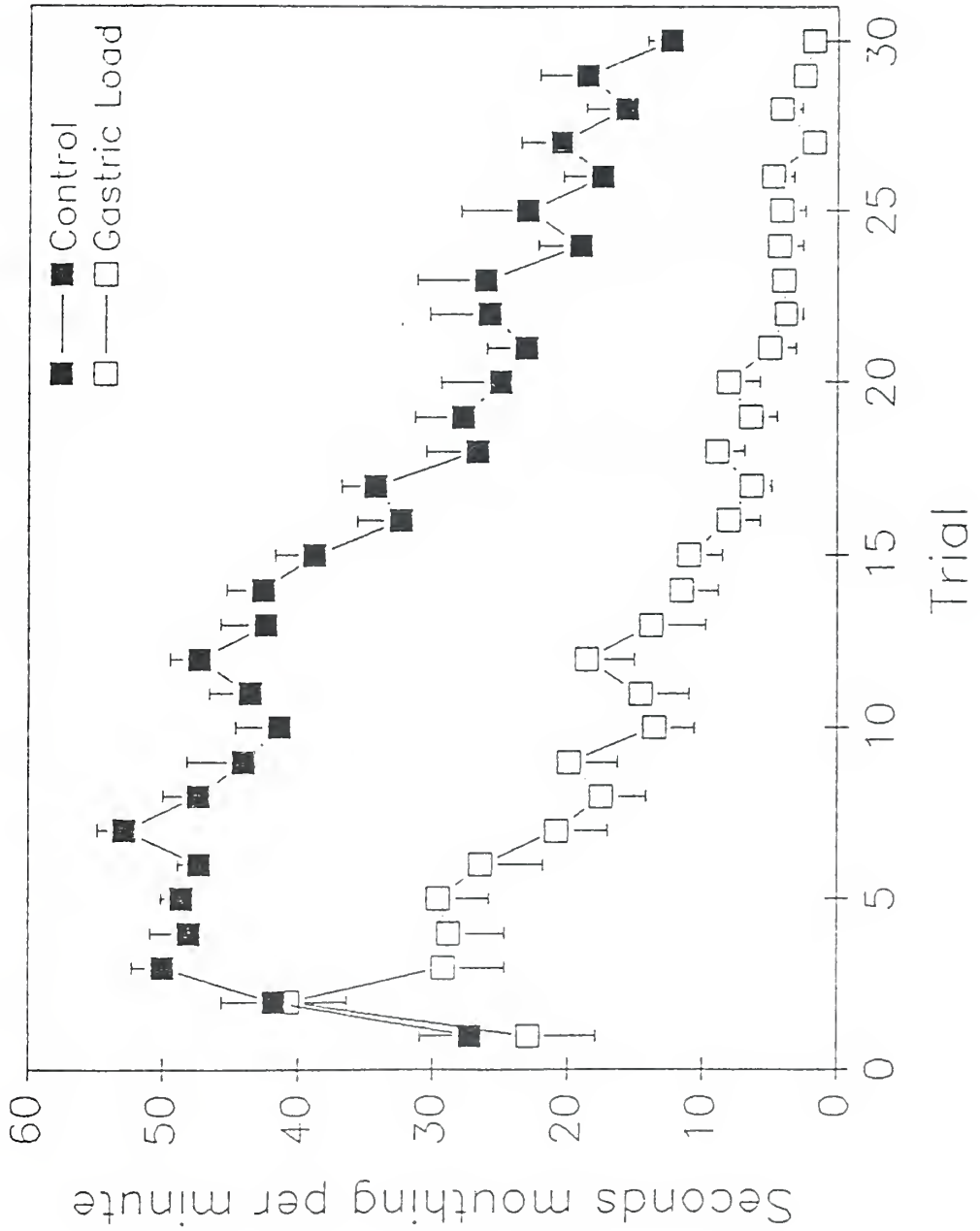


Figure 2.

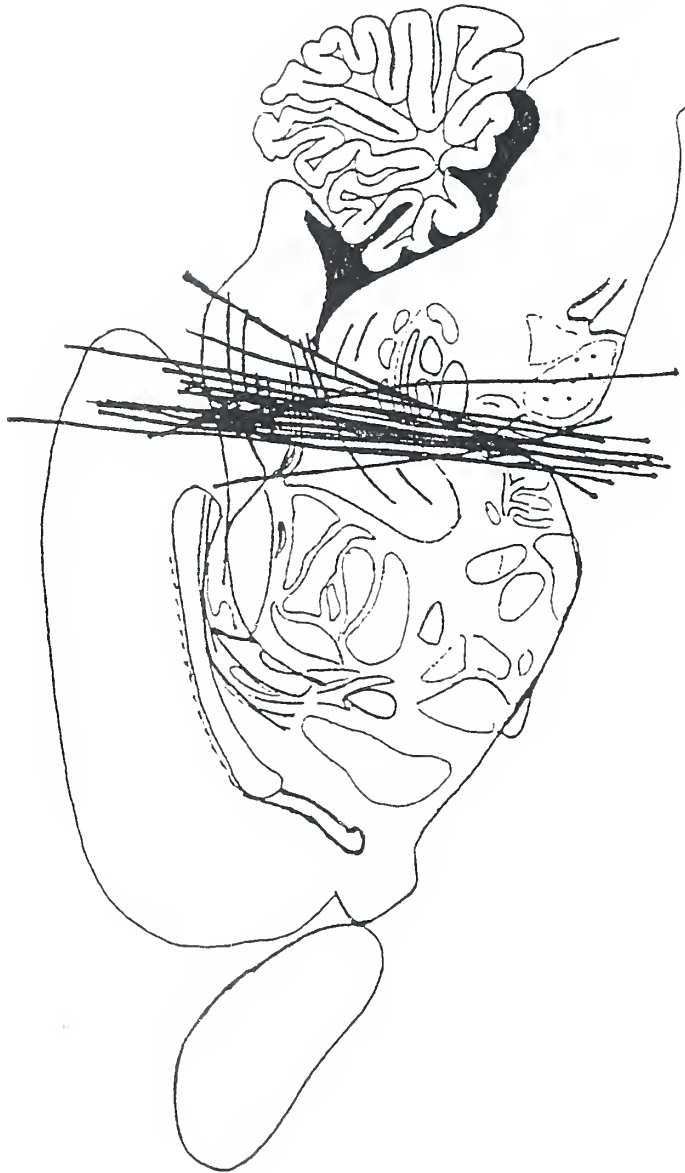
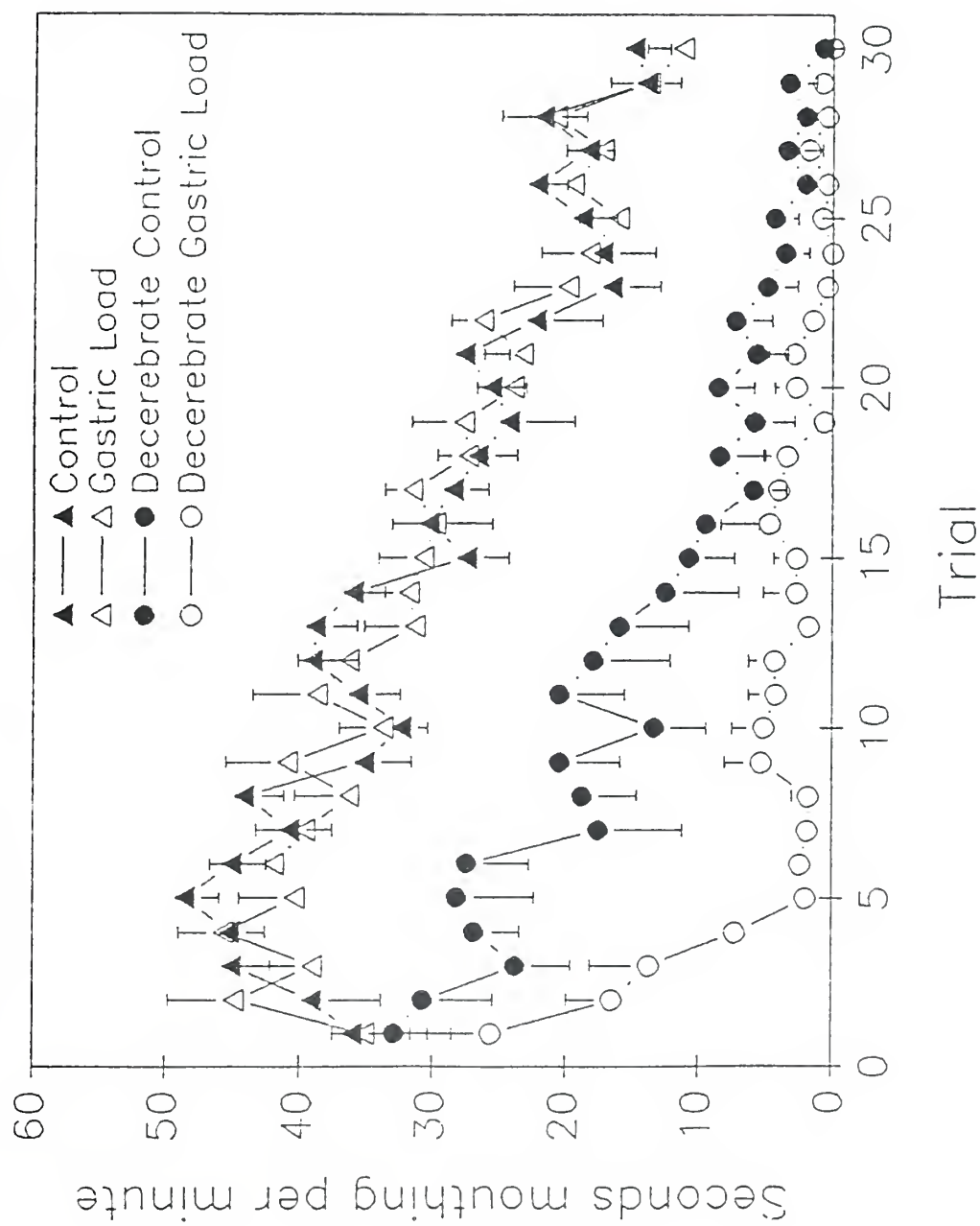


Figure 3.



CHAPTER VI.

ORAL EXPERIENCE CONTROLS INGESTION

ABSTRACT

In traditional considerations of the control of ingestive behavior, one or more postingestive signals is often attributed a major role. Other perspectives suggest that associative learning processes are largely responsible for the determination of ingestion. In this paper, we present an alternative view, that an oral, experience-based mechanism may be the primary control that terminates ingestive bouts and upon which other ingestion related signals exert their influence. Using data from studies of ingestive behavior in developing rat pups we demonstrate that oral experience alone, separate from postingestive influences, has profound effects on subsequent ingestive behaviors. In response to repeated oral stimulation, the oral activity of rat pups declines in a habituation-like manner, and habituation of oral activity significantly suppresses both short-term and longer-term intake. Oral habituation is sensitive to physiological signals related to gastric fill and deprivation state. Further, the expression of oral habituation and some of its integrative capacities is mediated at a brainstem level or lower; decerebrate pups show habituation-like decreases in ingestive behavior following repeated oral stimulation and these decreases are modulated by gastric fill signals. When taken together with previous demonstrations in adult rats of the necessity of oral participation in ingestive behavior to explain normal intake, these results suggest that oral habituation play an essential role in the control of ingestion.

The oral experiences that are generated by the act of ingestion have long been assumed to play some role in terminating an episode of ingestive behavior. In many theories of feeding control, an inhibitory signal produced by eating and conveyed through oropharyngeal receptors is considered to be one of several negative commands that are summed to create satiety. But although an oropharyngeal role in controlling ingestion has often been recognized in this manner, the contribution of oral experience is usually accorded only a small weighting in the overall equation of ingestive control. And even this small allotted role has been generally neglected in deference to the analysis of more mechanistically captivating physiological control signals. Using suggestive data from developmental studies, we argue a different perspective here: that an oral, experience-integrating mechanism may constitute the fundamental control that universally terminates ingestive episodes and that integrates other ingestion-related feedback signals.

Phenomena Revealing "Oral Factors" in Feeding Control

Consider some of the well known phenomena that already implicate the mouth in ingestive control. The first of these, one of the most thoroughly acknowledged but poorly reconciled findings in the ingestive behavior literature, is the fact that when the mouth is bypassed and an animal is provided intragastrically or systemically with all or a portion of its required nutrients, subsequent ingestion is neither eliminated nor reduced to the full extent of the load. Participation in ingestion by an animal seems to be necessary for nutrient delivery to have its full intake-terminating or satiating effect. Numerous nutrient-loading experiments of this type have shown (see review, Kissileff & Van Itallie, 1982), as in the classic cases of Kohn (1951), Nachman and Valentino (1966), Miller (1957), and Berkun, Kessen and Miller (1952), that food "taken normally by mouth produces an even greater reduction [in hunger than food injected directly into the stomach]" (Berkun, et al., 1952).

Results indicating an importance of oral participation in ingestive control are particularly impressive in preparations such as parabiotic rats (e.g. Koopmans, 1978), in which nutrient delivery bypasses the mouth through a relatively physiological digestive and absorptive process. In these types of preparations the finding of a necessity for oral experience cannot be attributed to extraneous variables such as features of the loading procedure. When one rat of the pair receives no oral stimulation but still receives absorbed nutrients as a result of the ingestive behavior of the other rat, subsequent feeding by this first rat is typically reduced but not abolished. The test rat is sensitive to the nutrients it has received, but this sensitivity alone is not sufficient for the production of satiety.

A particularly paradigmatic example of this type, showing the importance of the role of oral participation in feeding control, was recently provided by Andreadis and Burghardt (1991). They described the feeding behavior of a congenitally two-headed snake in which both heads and brains shared a common gastrointestinal and metabolic system (Figure 1a). In this snake, the attack and swallowing of a mouse by one of the heads, which produces a postprandial lethargy and anorexia lasting days, did not prevent the second head from carrying out its own search and swallowing of a second mouse (Figure 1b). Thus, in this reptile oral participation was important to establish satiety.

A second type of phenomena pointing to the importance of recent oral experience in controlling ingestion is the effect of food variety on ingestion. An animal that has satiated itself on one diet is often willing to consume another, at times ingesting the same number of calories of the second diet before terminating ingestion. This phenomena in humans, commonly known as the "dessert effect," has been termed and studied as "sensory specific satiety" (Clifton, Burton & Sharp, 1987; Mook et al., 1980; Rolls et al., 1981a,b; 1982). The specificity of satiety for a particular diet makes several important points. First, the general physiological or metabolic effects of ingestion are not of absolute importance nor

independent of a food's significance as a sensory experience. Second, the mouth's role is more than that of a passive counter or meter; in some fashion the specific features of the ingestive experience are being recorded and exerting an influence on behavior.

In short, the well appreciated characteristics of the oral component of ingestion are that: a) **the mouth needs to participate** in nutrient acquisition for ingestive responsiveness to be quenched; and b) in its participation in ingestive control **the mouth expresses a specificity** for the ingestive experience it has undergone.

Yet Oral Factors Are Neglected

Despite these clear demonstrations of the necessity of oral participation and oral experience for the normal expression of ingestive control, and despite the periodic attention that the mouth and oral sensorimotor processes have received, the oral control system has been viewed as only one of several signals contributing to the production of satiety. The 'oral factor' or oral control is usually allotted some fractional degree of "fine-tuning" in intake control (e.g. '1/5th,' cf. Kissileff & Van Itallie, 1982) in systems that simply sum multiple feedback signals. In such views, the oral contribution is typically one of many signals directed to a central integrator (e.g. Figure 2). While not completely ignoring the role of the mouth, most hypotheses for feeding control, being heavily influenced by the remarkable homeostatic achievements of the energy balance system, have extolled the role of one or another feedback signal arising from the post-ingestive consequences of ingestion. At best, the oral factor is accorded the same additive roles as these peripheral factors, but more typically receives little consideration.

The attractiveness of potential physiological and metabolic mechanisms as controls of ingestion is one reason for the lack of attention to oral mechanisms. A more significant reason is that findings from 'sham feeding' and 'sham drinking' studies seem to indicate that

oropharyngeal activity is not itself sufficient for the production of satiety (Young et al, 1974). Sham feeding rats, animals in whom the ingested food does not reach its normal postingestive sites, have been found to feed continuously for hours (see typical curve, Figure 3). We believe there are problems with the interpretation these of sham-feeding data and we will return to them in a later section. Note for the moment, though, that while oral factors may not in themselves be sufficient for terminating ingestion, they may still be essential -- potentially in ways that other factors are not. The concept of an additive role for the mouth, as conceived in most models of ingestion (e.g. Figure 2) does not explain the necessity for some oral participation in the termination of ingestion, a necessity that exists even when there is a surfeit of other inhibitory signals. Given the significant interest in understanding the control of ingestive behavior, it seems ironic that explanations for the control of the oropharyngeal apparatus frequently fail to take into account the potential role of activity of the oral sensorimotor system itself.

A Developmental Perspective

In this paper we review a series of recent experiments (Swithers-Mulvey & Hall, 1991a, b; Swithers-Mulvey, Miller & Hall, 1991; Swithers-Mulvey, Mishu & Hall, 1991; Swithers-Mulvey, Westneat & Hall, 1991) that reveal an unappreciated importance of oral factors in ingestive control and that suggest how a mechanism for encoding ongoing oral experience during ingestion may serve as the integrator for other ingestive controls. Our perspective on oral experience emerges from a developmental analysis of ingestion in rodents. The techniques utilized in this analysis provided for unusually explicit control of oral presentation of ingestive stimuli. It will be seen that the use of developing animals along with this controlled presentation of oral stimuli combined to expose the role for oral experience in ingestive control.

In the studies we review, oral experience with a diet was explicitly programmed by providing young rat pups with a series of brief intra-oral infusions of sucrose or saccharin solution through indwelling oral cannula (Figure 4). Such infusions are avidly ingested by young rats, and it is well established that this ingestion is under the pup's control -- pups must make active licking, lapping and swallowing movements or the diet will spill out of their mouths. With these infusion procedures, ingestion is like that of free-feeding adult rats to the extent that intake volumes are influenced by pups' physiological state (e.g. Hall & Bryan, 1980).

To increase the likelihood of detecting contributions of oral experience we sought to minimize the contributions of post-ingestive effects in our tests. We made the duration of each programmed infusion brief, and the volume delivered quite small. This minimized the amount of gastric filling. During the entire experiment, pups received only a fraction of the amount of diet they would have consumed if they were allowed to freely ingest a diet which was continuously infused. Other post-ingestive signals were effectively eliminated by the use of sucrose or saccharin as the infused diet. Because young rat pups have little or no sucrase enzyme activity, they do not readily digest or absorb sucrose solutions. For experiments lasting several hours, where sucrose diets might have led to dehydration, we used saccharin solutions which pups also readily ingest and which are non-nutritive.

To assess oral responsivity, we recorded the duration of oral responding, as evidenced in mouthing activity, to each infusion in the series. Thus in these experiments, each brief infusions served both as a stimulus that provided oral experience with a diet and as a probe of oral responsivity to that diet, indicated by amount of mouthing.

Oral Experience Reduces Oral Responding

When pups were tested in this manner, the effects of oral experience were

remarkable. Pups showed dramatic decreases in ingestive behavior; oral responsivity to these repeated stimulus presentations steadily decreases (Figure 5). Oral responding declined to a level of virtual non-responsivity and this occurred despite the fact that pups had ingested very little volume, that levels of gastric fill were likely to be low, and that the diet was not absorbed. The remarkable potency of this decremental effect of repeated diet exposures is rendered particularly impressive by the finding that in the 18-day-old pups depicted here the decrement was present in the face of extended food and water deprivation. Oral responding diminished to low levels with repeated stimulation even following deprivation periods as long as 24 hours. This reliable decline in oral responsiveness effected by repeated oral stimulation represents the fundamental phenomenon that this paper explores and extols.

We know that pups which are freely ingesting from puddles on the floor or from a continuous infusion will consume sucrose solutions to considerably greater gastric fill levels than we provided pups here (e.g. Hall & Bryan, 1980), thus it is unlikely that postingestive effects of the infused solutions, such as gastric filling, could be responsible for the profound decline in oral responsivity. Rather, the decline in responding appears to be a result of the oral experience with the diet. To completely rule out the possibility of a contribution of post-ingestive effects under these conditions, we carried out experiments in which a group of pups received identical paired infusions intragastrically for 30 min. Then during a second 30 min period, these pups received oral infusions. In this subsequent test of responding, the oral responding of pups that had received the first 30 infusions intragastrically was identical to that of pups which had received no infusion at all (Figure 6) with the decreased responding of both of these groups being similar to the decreased responding of the original oral experience group during the first 30 min. Thus, the repeated oropharyngeal experience with a diet, not the gastric or postgastric effects of the diet, produces a distinctly diminishing

responsiveness to the diet. In these experiments with pups, the experience of diet by mouth was decidedly more potent than diet into the stomach (Swithers-Mulvey, Miller & Hall, 1991).

The Decrement in Oral Responsiveness is Diet Specific

If the decrement in oral responding that occurs with repeated oral stimulation is an effect of the oral sensory or proprioceptive experience with the diet, as opposed to motor fatigue or a more general metering, it would be reasonable to expect some stimulus specificity. We tested the question of specificity by assessing whether the depressed response could be restored by changing the diet that was offered to the pups. If animals reduce responding because of a general monitoring of oral behavior or simply because their mouths are exhausted, then a new diet shouldn't change the level of responding. In these experiments, pups received oral infusions of a sucrose solution as described above, but the solution was now flavored with Grape or Cherry Kool-Aid. At 21 min, a point midway through testing, half the pups were switched to a different flavor diet while the other half continued to receive the same flavor solution.

Switching the flavor of the diet reinstated responding (Figure 7). The restored responding argues against general metering or fatigue as a cause of declining responsiveness and indicates that the oral experience effect is relatively specific (Swithers-Mulvey, Miller & Hall, 1991)

The Decrement in Oral Responding is Long-Lasting

The decline in oral responsiveness with repeated experience conceivably could be due to short acting sensory receptor effects. It might thus have a role in punctuating bursts of ingestion but have relatively little implication for influencing intake on more than a momentary basis. To assess the duration of the decremented oral responding following

repeated diet exposure we interposed a 30 min or 3 hr interval between an initial 30-infusion stimulus series and a subsequent infusion test for the persistence of the decline in responsiveness.

After a 30 min or 3 hr retention interval, pups that had received the previous oral experience continued to show a significantly reduced level of responsiveness relative to their own initial responding and relative to the responding of a group that had received no stimulation (Figure 8). This 'memory' of the ingestive exposure remained diet-specific both at 30 minutes and, though somewhat diminished in degree, at 3 hours; oral responding to a diet different from the one previously experienced remained high. The effects of experience that accrue from ongoing ingestive experience are thus potent and relatively long lasting, having persisting effects on ingestive responsiveness and thus potentially persisting effects on intake control.

Let's Call it 'Oral Habituation'

These patterns of declining oral responsiveness to repeated oral infusions resemble patterns of decrementing responding that have been described in many other behavior systems and in animals from aplysia to human, including rat pups. In these systems, decreased responding has been termed habituation. 'Habituation' is used descriptively to reflect a specific and relatively long-lasting response decrement resulting from repeated stimulation (e.g. Harris, 1943; Thompson & Spencer, 1966). The results described above indicate that, in developing rats, the mouth habituates to presentations of fluid stimuli.

Besides showing a distinct decrement that is relatively long-lasting, the course of oral habituation seen in rat pups parallels habituation described in other systems in several ways. First, while habituation is relatively specific in both cases, the distinct but incomplete restoration of responding resulting from switching diets resembles the results of other

habituation studies in which habituation has been shown to partially generalize to related stimuli (Thompson & Spencer, 1966). Here it is not surprising, given the similarity in sucrose content of the solutions which pups received, that there should be some generalization between stimuli. In fact, given the immaturity of pups' sensory systems (taste, olfaction) it is particularly impressive that they seem to discriminate between the two stimuli as well as they do.

A second parallel in these oral habituation data with traditional habituation studies is the observation of an increase in responding during the first several trials (e.g. Figure 7). Such an initial increase in responding in other studies is usually termed 'sensitization,' an increment in responsiveness that in many behavior systems has been shown to be independent of the habituation process and to generalize extensively to other stimuli and behaviors (Groves & Thompson, 1970). As well appearing at the beginning of a sequence of stimuli, sensitizing effects on responsiveness can frequently be produced by a single strong stimulus in other modalities (e.g. shock to the tail or head of *aplysia*; Pinsker et al., 1973). Thus, sensitization, like habituation, is a fundamental, though separate, property of behavioral systems. Like habituation, it constitutes a basic form of non-associative learning and as such represents one of the most fundamental manners in which experience can influence ongoing behavior. Sensitization is an operationally descriptive term which is consistent with the broader psychological constructs of arousal and incentive that are frequently used with regard to effects of oral ingestive stimulation. The sensitization process deserves more attention by those interested in feeding control and would be amenable to an experimental analysis such as the one we are describing for habituation. We will, however, save it for another discussion.

Some confusion about what we mean by habituation is not unlikely because the term habituation (as well as sensitization) are often imprecisely and casually used. Thus,

we would like to make it clear that we do not use 'habituation' to suggest a process occurring in the mouth at the receptor level; such a peripheral process might more accurately be termed sensory or receptor adaptation. Receptor adaptation and habituation are logically similar processes, but receptor adaptation refers to a decrement that can be attributed to peripheral rather than central processing and that characteristically is quite short-lived. Although both adaptation and habituation are likely to contribute importantly to the patterning of ingestive behavior (we return to this issue later), the finding that the decrement in ingestive behavior persists for at least 3 hrs rules out adaptation as a possible explanation for the suppressive effects of oral experience in the paradigm described above.

The term habituation can also be confusing because it has been used both to describe a behavioral process and to refer to potential mechanisms. While our ultimate interest may lie in understanding the cellular mechanism that produces response decrements, recognize that there is no reason to expect that the mechanism responsible for the process of oral habituation in rat pups is the same as that responsible for habituation in another system (e.g. for gill-withdrawal habituation in *Aplysia*) or, more importantly, that the process of habituation observed at one level of behavioral organization is subserved by the same mechanism as that at another (e.g. spinal cord, Groves & Thompson, 1970; brainstem, Davis & File, 1984; neocortex, allocortex & mesencephalon, Teyler et al., 1984). Indeed, mechanism remains an open question for most habituating systems.

We utilize the term habituation here with respect to oral experience because as a descriptor of a behavioral process it so well captures the characteristics of the system's intrinsic and fundamental properties. Although it is a general term and does not emerge from the ingestive behavior literature (in the way of a term such as 'oral metering,' discussed below), the concept of habituation, well explored behaviorally and neurobiologically, brings a significant history of theory, experimental strategy, and data to the present analysis.

Oral Habituation Suppresses Food Intake

The preceding description indicates that after repeated brief infusions of a diet, oral responding decreases dramatically, even when pups are nutritionally deprived. That is, oral habituation of mouthing responsivity occurs. But, is a decline in this measure of oral responsiveness relevant to controlling intake? If it is, it should have an impact on the amount of food an animal actually ingests and this effect on intake should be relatively long-lasting. To test the question of whether habituation of oral responsivity influences real intake, we gave pups a series of oral infusions of a flavored sucrose diet, and documented the decline in oral activity. Control groups received paired intragastric infusions of diet, or no infusions of diet. Then, following the initial series of infusions, pups were given one of two types of intake tests: i) one in which they were allowed to consume a diet infused continuously into their mouths for 4 minutes; and ii) a second in which pups were required to ingest test diets from the floor of their test containers for 21 min. The flavor of the diet during the ingestion period was either the same as during the initial series of infusions or was switched to a second flavor (Swithers-Mulvey, Miller & Hall, 1991).

Pups that received the same flavor orally in both the initial series of infusions and the ingestion tests consumed little of the diet and ate significantly less than all other groups (Figure 9). The suppression of ingestion by oral experience was specific to the diet experienced orally in both types of tests. Pups that received oral infusions of different diets in the initial infusion series and ingestion test consumed as much as pups that had received no infusions. This specificity was not post-ingestive; pups that had received intragastric infusions consumed the same amount in the ingestion test as pups that had received no infusions whether the diet ingested was the same as or different from the diet infused intragastrically.

To assess the longer-term effects of oral experience on intake, pups were given an

initial series of oral infusions of a flavored saccharin solution and a 4 minute continuous test infusion was delivered either immediately after this initial series, 30 minutes later or 3 hours later. Saccharin was used in these experiments because of concerns of the potential dehydrating effects of non-digested sucrose in the longer-term studies and to confirm that oral habituation was not peculiar to sucrose solutions.

As seen with sucrose infusions, an oral habituation to the initial series of saccharin infusions was expressed. This oral habituation reliably decreased subsequent ingestion when tested immediately, and this immediate suppression was diet specific. Diet-specific suppression was still evident 30 minutes later. Even after 3 hours, ingestion was significantly suppressed compared to pups which had previously received no infusions of diet (Figure 10). However, the specificity of suppressed intake had largely worn off as intake was not different between groups that received the same or different flavors during habituation and intake. The diet-specific intake suppressing effects of oral habituation thus last at least half an hour and more general suppressive effects remain for at least 3 hrs providing a potential for habituation to influence both intra- and inter-bout ingestive behavior.

These data from developing rat pups reveal an impressive potential of the mouth in controlling ingestion. The oral habituation process provides an animal with an experienced-based control of intake; a straightforward way to influence its present ingestive behavior based on ongoing experience. Habituation allows the intrinsic effects of the oral experience generated by sensory and proprioceptive events of eating to be readily appreciated by the central nervous system. Stated in another manner, responses habituate with 'use' and the degree to which they habituate influences their subsequent expression. Although the role of the mouth is not usually viewed by psychobiologists from a habituation perspective (though see Thorpe, 1966, and other ethological perspectives, e.g. Tinbergen, 1951, for suggestions that feeding control originates with the behavioral regulation provided by habituation), the

concept of habituation provides a way of conceptualizing what has been meant in psychobiologists' reference to the "oral phase" or "oral factor" in feeding control, a control that has also been termed "oral metering". As Mook (1991) has expressed it " (oral metering is).. how the rat's body tells the rat's brain what's going on."

The Mouth as Integrator

It is an important first step to recognize that the oropharyngeal-sensory-motor systems for ingestion have a habituating property. But the significance of the habituation property of ingestive systems goes beyond just explaining how oral factors help inhibit ingestion. Simultaneous with its experience-encoding role, habituation is a process that occurs over time and that has several parameters available for modulation by signals arising during the course of ingestion. Because these parameters can influence subsequent intake and the course of satiety, the oral habituation process is able to serve as the integrator of postingestive physiological signals, expressing the effects of postingestive consequences of consumption in ongoing behavior. The parameters of the habituating response system available for modulation include at least the following: a) its initial level of responsiveness; b) its rate of decrement in responsiveness to oral stimulation; and c) the specificity and duration of the decremented responsiveness. If any or all these properties of the consummatory response are influenced by postingestive physiological signals (e.g. those related to gastric fill, metabolic state, hydrational state, etc.), we are provided with an effective way for the consequences of ingestion to be integrated into ongoing behavior, determining within-bout and meal-to-meal characteristics of consumption. As an integrator, habituation thus potentially provides a use-sensitive mechanism, intrinsic to the response system, that can serve an animal as a real-time interface for its physiological state and recent feeding history and a way of expressing these in ongoing behavior by controlling oral

responsiveness. To put it simply, whenever ingestion terminates the cause may be oral habituation, with postingestive consequences serving to alter the rate of habituation and the duration of the habituation effect. Moreover, because influences on the parameters determining responsiveness may be broadly sensitive to subtle features of physiological state, habituation, more broadly, could be the proximate mechanism for effecting long-term regulation of energy balance and body weight. In short, the mouth and its inherent habituating nature may be the focal integrator for control of ingestive behavior. In the following sections we explore further this potential integrative role of the mouth, provide suggestive data regarding the influence of physiological signals on the system and its neural substrates, and consider additional questions that will be important in resolving the full contribution of oral experience to ingestive control.

How Physiology Has Its Influence

We have evidence in developing rats that oral habituation parameters can be affected by physiological signals. The influence of gastric fill was assessed in 12-day-old pups after 6 hours of deprivation, an interval designed to produce empty stomachs by the time of testing. Pups were tested with a series of brief oral infusions as described above. Beginning at the same time as the start of the first oral infusion, pups received a continuous gastric load of sucrose delivered over a period of 5 minutes through indwelling gastric cannulas. The volume loaded gastrically was equal to approximately 5% of the pup's body weight, and constituted an appreciable amount of gastric fill. A control group received only oral infusions. The mouthing activity of both groups was continuously recorded. During the minute following the first oral infusion, response levels were similar in control and gastrically loaded pups, thus initial rate appeared unaffected by the concurrent gastric load (Figure 11). Following the second oral infusion, evidence of sensitization was similar in the increased

responsiveness of both groups. However, beginning with the third oral infusion, the mouthing responses of gastrically-loaded pups began to decline more rapidly than the responses of control pups and remained lower throughout the duration of testing, even beyond the time of the delivery of the load. Thus, in this situation, oral habituation rate is modulated by gastric filling. The habituation process is sensitive to the postingestive physiological signal and appears to integrate it with accruing information about oral experience (Swithers-Mulvey & Hall, 1991b).

We also expected that the nutritional and hydrational deficits produced by deprivation to influence habituation parameters, but were surprised by the results when we explored this question developmentally. In young animals, deprivation did have noticeable effects. In fact, 6-day-old pups tested in a habituation paradigm following 24 hrs deprivation demonstrated greatly elevated response levels from the outset and failed to exhibit appreciable habituation to repeated stimulation (Swithers-Mulvey, Miller & Hall, 1991). More mildly deprived 6-day olds also showed higher initial response levels compared to non-deprived controls, but their mouthing activity habituated, like that of controls (Figure 12A). These results thus confirmed the potential of effects of initial state on habituation parameters. In older animals, the same deprivation levels had less marked results. Twelve-day-olds deprived for 6 or 24 hrs prior to testing both showed increased initial responding compared to non-deprived pups and both habituated (Figure 12B). In fact, the responses of 6- and 24-hr deprived pups were indistinguishable from one another. So at 12 days of age, there seems to be some effect of 6 hrs deprivation, but increasing the length from 6 to 24 hrs has no additional effects. Finally, by 18 days of age, even 24 hrs deprivation had little or no effect on the course of oral habituation (Figure 12C). Pups deprived for 6 hrs, 24 hrs or tested non-deprived at 18 days of age demonstrated similar patterns of habituation. Several possible explanations underlie this developmental change in the effects of

deprivation on habituation. First, the effects of deprivation in young pups may reflect an unusual sensitivity to hydrational and maternal care deficits. Another possibility is that the same length of deprivation in pups of different ages do not result in similar physiological states and thus we might have seen an effect in older animals with greater deprivation. Whatever the cause, the data from pups at all ages certainly show the potency of the oral habituation effect over the ingestive drive, decreasing oral responsiveness even in the face of extended deprivation.

Despite the fact that continued oral habituation in 24-hr deprived 18-day olds strongly argues the effectiveness of repeated oral stimulation in reducing ingestive behavior, the apparent lack of a deprivation effect in 18-day olds was initially quite troubling. Given that we know that intake is altered by deprivation at this age, we had expected that the increased intake might be accomplished by an increase in oral responding or a direct effect on the rate at which oral activity habituated. However, an examination of the combination of deprivation with gastric fill revealed a mechanism for an influence of deprivation on the oral habituation process that may explain the effects of deprivation on actual intake. As described above, in 12-day-old pups, 6 hrs deprivation results in an higher initial response levels compared to non-deprived pups but increasing deprivation length to 24 hrs has no additional effects. Further, the presence of appreciable gastric fill signals along with oral stimulation in a 6-hr deprived 12-day-old pup results in more rapid oral habituation; pups stop their oral activity more rapidly and more completely when gastric signals accrue (Figure 11). In contrast, these same gastric signals have little or no effect on oral habituation when delivered to 12-day old pups deprived for 24 hrs. The rate of oral habituation in 24-hr deprived 12-day-olds is insensitive to level of gastric fill (Swithers-Mulvey & Hall, 1991b; Figure 13). So while increasing deprivation does not by directly enhance initial activity of the oral habituation system or its rate of decrement *per se*, increased deprivation does

change the manner in which at least one other physiological signals, gastric fill, affects oral habituation. Such suggestive demonstrations illustrate a compelling capacity of the oral habituation system to integrate multiple state-related signals with ongoing ingestive experience.

The Neural Site for the Ongoing Integration of Ingestive Controls

The hindbrain is the logical site for the substrates of at least a portion of the oral habituation process. The basic oral sensorimotor loop as well as second order projections and interneurons are present in the pons and caudal to it. This represents an adequate circuitry for habituation. Moreover, habituation substrates for other responses (e.g. Groves & Lynch, 1974; Jordan, 1989) are present in such caudal sites. In addition, for ingestion, research with decerebrate rats has revealed that many of the features of consummatory responding are present in the hindbrain including taste preference and aversion, and gastric fill effects on intake (Grill, 1980; Grill & Norgren, 1978). As a starting point to understanding more about the characteristics and basis for oral habituation, we have made a preliminary analysis of the effects of decerebration on the oral habituation process we have described in rat pups.

To assess the oral habituation capacities of the brainstem, pre-collicular transections were made in a group of 12-day-old pups and their responses to a series of oral infusions of sucrose were recorded following a 24 hour recovery (and deprivation) period. The results clearly demonstrate that even in the absence of forebrain influences, mouthing responses habituate (Swithers-Mulvey, Mishu & Hall; Figure 14). Further, decerebrate pups retain the capacity to integrate gastric fill signals (Figure 15); decerebrate pups which received a gastric load equivalent to 5% of their body weight showed more rapid habituation than decerebrates receiving no gastric load (Swithers-Mulvey & Hall, 1991b). Thus, the neural

substrate for the production of oral habituation and encoding the habituation memory as well as its response to at least one physiological signal is present in the hindbrain in pups and does not require forebrain participation.

However, note that in the data presented in these figures, a forebrain contribution to oral responsiveness is clearly revealed in the differences in behavior observed between neurologically intact and decerebrate pups. While both intact and decerebrate animals show initially high levels of responding, the activity of normal animals starts somewhat higher and increases over the first trials, while the oral responses of decerebrate animals immediately decline. Thus the sensitizing or arousing properties of oral stimulation appear to be dependent on forebrain influences.

Moreover, we discovered an intriguing difference between decerebrates and normals in assessing the effects of deprivation. As described above, 6-hr-deprived, normal pups respond to gastric loads with a more rapid rate of habituation, whereas the same normal pups deprived for 24 hours fail to alter rates of habituation in response to gastric fill. Decerebrates, on the other hand, do demonstrate more rapid habituation in response to gastric fill, even when tested following 24 hours deprivation (e.g. Figure 15). These results suggest that influences of deprivation state depend on intact communication with the forebrain. That is, brainstem mechanisms seem sufficient to subserve oral habituation and the gastric fill control of habituation rate, but that further modulatory effects on the potency of gastric fill control are exerted by deprivation through forebrain systems.

Adding Temporal Dynamics to the Equation: The Curious but Telling Effect of Varying Stimulus Timing

In the experiments described thus far, we have shown that oral experience, by virtue of a habituation mechanism, can provide a potent control of ingestive behavior -- a control

capable of integrating postingestive signals and a control whose mechanisms seem largely present in the hindbrain. As we indicated at the outset, detection of such clear effects is a result of two features of our experimental paradigm, stimulus presentation control and developmental analysis. To place this work in broader context, these two features of the analysis are considered in this and the next section.

Programmatic presentation of stimuli, a standard habituation analysis technique, allowed us to detect potential experience effects on ingestive control. Yet, such discrete and controlled stimulus delivery is quite different from the stimulation that occurs during normal ingestion in which an animal initiates consumption and sampling of diet according to its own pattern. The normal timing of experiences with food stimuli can thus be highly variable. Other habituation systems have been shown to be very sensitive to the timing of stimulus presentation (e.g. Davis, 1970). For this reason it is likely that under normal feeding conditions, the variable rate of stimulus presentation (or length of inter-stimulus interval; ISI) might produce varied habituation effects. Conveniently, use of a habituation paradigm permits a controlled assessment of the importance of the ISI on the habituation produced by oral experience. In particular, more continuous ingestion can be modeled with smaller intervals between stimuli. We therefore examined the effects of varying interstimulus intervals on the expression and duration of oral habituation.

In these tests, one group of pups received oral infusions of sucrose once every minute, as in all of the tests described above. A second group of pups received the same number of oral infusions but more rapidly - once every 20 seconds. Thirty minutes later (relative to the last infusion for each group) pups received a 4 minute continuous oral infusion and the amount of this infusion that they consumed was measured. Our interest was in both comparing the pattern of responding to the repeated stimuli with the two different intervals, assessing whether a similar decrement occur in both cases, and in

comparing the duration of the memory for the oral habituation experience.

When the duration of mouthing was expressed as a percentage of the interstimulus interval, the decline in oral responsiveness showed similar patterns to stimuli presented at both rates (Swithers-Mulvey & Hall, 1991a; Figure 14A). Both groups showed distinct and similar decreases in oral activity. In fact, in absolute terms, the short-interval group showed a greater depression than the 1 min interval group. However, when intake was assessed 30 minutes after the last infusion, suppression of intake was seen only in the 1 min interval group (Figure 14B). The group which got more rapid stimulus presentations showed little effect of the prior oral experience. These pups ate as much of the diet as animals that had not received any infusions. These data show that when ISIs are shortened there is a decremental process that modulates oral responsivity but in this case the effect is quite short-lasting, perhaps being attributable to peripheral adaptation-like processes.

The startling difference in duration of the decrement in oral responsivity with these two different ISI's reveals an intimidating complexity to the oral experience mechanism. It suggests that during normal ingestion, both short-lasting decrements (i.e. resulting when there is almost continuous ingestion) and long-lasting decrements (i.e. resulting when there are pauses between episodes of ingestion) combine to generate the patterning of ongoing behavior. Indeed, short-term decrements in responding may create the pauses in ingestion (i.e. between stimulations) that permit long-term decrements to occur. Short-term and long-term phenomena are conceptually similar and can be viewed as logically-related processes acting at different neural levels (e.g. Sherrington, 1947; cf. modern conceptualizations of multi-stage processing, e.g. Wagner, 1976). But because they have a different time-base of persistence for effect, their contribution to control can be quite different and the dynamics of the interaction complex. Moreover, identifying a relatively short-term (i.e. lasting less than 30 min) and longer-term process does not preclude additional processes with other time

bases -- including the possibility of long term effects persisting for many hours to days produced by repeated habituation experiences.

Studying these oral experience processes using the temporal control provided by brief oral infusions is a direct tool for exploring and defining the characteristics of their mechanisms. Without such stimulus control, it would be impossible to appreciate the temporal dynamics of such mechanisms or to unconfound the complexities resulting from the interactions of their temporal interdependence. Clearly, an advantage of the habituation paradigm approach is that it allows explicit mapping of stimulus dynamics.

The Mouth in the Feeding Sequence

The second feature of our analysis which has contributed to detecting oral experience effects is developmental analysis. In young pups, the appetitive component of independent ingestion has not yet emerged (e.g. Hall, 1990). Thus it has been natural/convenient for experimenters to study ingestive ontogeny by studying control of the consummatory response and to elicit consummatory responses with oral infusions or other techniques that minimize the requirements for appetitive behavior. On the animal subject's part, pups come to the test situation with little ingestive experience. Their experience with ingestion in the suckling situation is unlike that of adult-like, independent ingestion (e.g. Hall & Williams, 1983). Pups have had no oral experience with the specific diets with which they are tested nor with the postingestive consequences of these diets. Thus ingestion of oral infusions is unlikely to be under any learned control. Finally, because pups are not experienced with the normal appetitive-consummatory sequence of ingestion, tests with oral infusions do not violate an expectation with respect to the normal sequence of events in feeding, as they can do in adults (Hall & Smith, 1990). Indeed, at this point we know little about the relationship of habituation of the consummatory response and appetitive

components of the ingestive behavior sequence.

We believe that the mechanisms of control of ingestion by oral experience that have been defined in young rats are also functional and fundamental in adults, however we have not yet studied oral experience effects in adults with the procedures described here. Because of the factors just discussed, detecting and demonstrating what may be a primary and integrative role for oral habituation in adults may not be straightforward and will require thoughtful strategies. In particular, the effects of previous learning about ingestive stimuli or ingestive consequences will confound analysis in adults (cf. Booth, 1977; Weingarten, 1985). Perhaps more importantly, in adults it is difficult to separate appetitive from consummatory components of ingestion.

These difficulties are reflected in experiments with 'sham feeding' in adult animals, a classical methodology designed to assess the 'oral' processes in ingestion. Sham feeding has been viewed as a test of the oral component because during sham feeding all of the food an animal eats (ideally) spills out of a fistula in either the esophagus or the stomach. But the sham feeding procedure overlooks the fact that what the mouth does is express the last portion of the ingestive behavior sequence. A simple illustration demonstrates why experimental isolation of behavioral components is important. Suppose an animal that is sleeping or grooming or simply sitting in the back of its cage. Before it can eat anything, it must become activated or aroused. Then it needs to locate, identify and approach a food source. Only then does the oral apparatus come into play. If an animal doesn't eat, we don't know whether it doesn't eat because it wasn't awake or because its mouth had habituated. The question of whether various components of ingestive behavior are controlled by the same signals or mechanisms remains open. But it would seem reasonable that oral habituation may be relatively independent of whether an animal is awake and searching for food. In fact, there are data indicating that at least some components of

ingestion may be differentially controlled.

A further complication to the interpretation of sham feeding experiments in adults has been the use of animals that are highly experienced. In fact, these studies are often carried out only after animals have begun to 'sham feed' persistently at high rates (e.g. Mook et al., 1983). While this acquired pattern of sham feeding has been thought to reflect an 'unlearning' of previous associations between a food and its post-ingestive consequences (e.g. Weingarten & Kulikovsky, 1989), recent evidence indicates that adult rats 'learn' to sham feed even when they have no previous experience with a particular diet (Mindell et al., 1990). Regardless of whether animals are 'unlearning' previous associations or learning that sham feeding is an unusual situation, the pattern of sham feeding is vastly different in an experienced sham feeder than in a naive one. Thus, which behaviors most closely resemble the 'normal' feeding situation remains undetermined.

The final difficulty with the volume of the sham feeding literature is also an interpretive one. Results from sham feeding data are often used to declare that oral processes play a minor role in the control of ingestive behavior because oral stimulation is insufficient for production of satiety. What this perspective overlooks is the fact that patterns of sham intake bear a remarkable resemblance to oral habituation and actually indicate that intake decreases dramatically over time in sham feeding animals, even when tested following lengthy deprivation periods (e.g. Gibbs & Falasco, 1978; Figure 17). From such a habituation perspective, it is also unnecessary to invoke associative processes to explain the 'acquisition of the sham feeding pattern'. Indeed, the increases in intake during successive experiences with sham feeding may simply reflect increased initial response levels and heightened sensitization (Gibbs & Falasco, 1978; Figure 17). From these heightened levels, the rate of habituation during sham feeding remains considerable and consistent across trials.

A Revised View of Oral Contributions to Satiety

The mouth no doubt contributes in some way to the production of satiety. Scores of studies of ingestion have illustrated this, whether it be showing that manipulations by-passing the mouth never fully explain control of intake, by the significant declines in oral-experience induced ingestive behaviors of sham-feeding animals lacking postingestive signals other, or by the demonstrations that satiety is specificity to diets actually ingested. However, previous considerations of feeding control have attributed only a small role to oral factors in deference to more compelling physiological signals like blood glucose levels. The results from the study of oral controls of ingestion in rat pups suggest that this disregard for the contributions of oral experience may be unfortunate. The data summarized in this review demonstrate that oral experience, in the absence of significant postgastric consequences, potently affects subsequent ingestive behavior and this oral experience-based can have long-lasting effects. Further, the oral habituation described here is responsive to other ingestion-related physiological signals, including gastric fill and deprivation, and thus may serve as the elemental process that integrates physiological state with ongoing behavior.

FIGURE CAPTIONS

Figure 1.

A. Two heads of a dicephalic snake shown fighting with each other to consume a mouse (from Burghardt, 1991).

B. Radiogram of snake showing labelled mouse in gastrointestinal tract and mouse recently swallowed by the left head.

Figure 2. Model of control of feeding behavior from Booth, 1978. Note that "sensory qualities of food" is but one of a number of controls integrated to control intake.

Figure 3. Typical cumulative intake curves of rats tested sham feeding glucose solutions (from Mook et al., 1983).

Figure 4. Drawing of 6-day-old pup with a cannula implanted sublingually in the front of the mouth. Cannulas are made of thin polyethylene tubing, the implantation of the cannula and its presence in the pup's mouth appear to have few aversive effects. An infusion of diet made through the cannula must be actively licked, lapped and swallowed or will passively drip out of the pup's mouth. In response to the infusions of sucrose described in this paper, pups show active mouthing behaviors consisting of movements of the jaws and tongue which continue after the infusion has ended.

Figure 5. Patterns of mouthing response to 3 sec infusions of 10% sucrose presented once each minute in 18-day-old pups. Mouthing responses were recorded continuously during testing and are represented here as averages seconds mouthing per trial over blocks of three trials. Pups were tested after 24 hrs or 6 hrs deprivation or tested non-deprived.

From Swithers-Mulvey, Miller & Hall, 1991.

Figure 6. Effects of intragastric infusions of diet on patterns of mouthing in 18-day pups. Pups in group Oral/Oral received a 3 sec oral infusion of 10% sucrose once every minute for 60 minutes. Pups in group Gastric/Oral received paired 3 second intragastric infusions of 10% sucrose through indwelling gastric cannulas once every minute during the first 30 minutes of testing. During the second 30 minutes of testing, the gastric infusions were stopped and pups received an oral infusion of sucrose once every minute. Pups in group None/Oral received no infusions during the first 30 minutes of testing and oral infusions during the second 30 minutes. From Swithers-Mulvey, Miller & Hall, 1991.

* $p < 0.05$ compared to group Oral/Oral

Figure 7. Effects of switching flavor of infused diet in 18-day-old pups. During the first part of testing, pups received a series of brief infusions of a flavored sucrose (0.05% Grape or Cherry Kool-Aid in 5% sucrose) solution. During the second half of testing, group Same continued to receive the same flavored solution and group Switch were changed to the other flavored diet. Changing flavors of diet significantly restored mouthing behavior to levels not different from initial responses. Note also the initial increase in mouthing behavior to the flavored sucrose diets in both groups over the first several blocks of trials in the first half of testing. From Swithers-Mulvey, Miller & Hall, 1991.

* $p < 0.05$ compared to Same.

Figure 8. Duration of mouthing behavior to a test probe delivered immediately following habituation or after a delay of 30 minutes or 3 hours. Test probe consisted of an infusion of diet for 60 seconds.

Figure 9.

A. Intake (mean \pm SEM) in 12-day-old rat pups of a 4 minute continuous infusion of a flavored (0.1% Grape or Cherry Kool-Aid) 5% sucrose solution tested immediately following habituation experience. From Swithers-Mulvey & Hall, 1991a.

* $p < 0.05$ compared to respective group None

$p < 0.05$ compared to respective group Oral/Switch

B. Intake in 12-day-old rats of a flavored 5% sucrose solution from the floor of test containers during a 21 minute consumption test. From Swithers-Mulvey & Hall, 1991a.

* $p < 0.05$ compared to group None

$p < 0.05$ compared to group Oral/Switch

Figure 10. Intake of a 4 minute continuous infusion of a flavored saccharin (0.05%) solution immediately following habituation or at a delay of 30 minutes or 3 hours. From Swithers-Mulvey & Hall, 1991a.

* $p < 0.05$ compared to group None

$p < 0.05$ compared to group Oral/Switch

Figure 11. Patterns of decrementing mouthing activity (means \pm SEM) in 6-hr deprived 12-day-old pups in response to a series of oral infusions of 10% sucrose. Each point represents the number of seconds mouthing per minute in each trial. Control pups received oral infusions only while Gastric Load pups received both oral infusions and a 2ml continuous infusion of 10% sucrose through indwelling gastric cannulas over 5 minutes starting at the same time as the first oral infusion. Beginning with the third infusion, the responses of gastric load pups were significantly lower than control pups. From Swithers-Mulvey & Hall, 1991b.

Figure 12. A. Effects of deprivation in 6-day-old pups. Each point represents the average number of seconds mouthing per trial over three trial blocks. Prior to testing pups were deprived for 6 or 24 hrs in a warm, humid incubator or were tested non-deprived. From Swithers-Mulvey, Miller & Hall, 1991.

* $p < 0.05$ compared to non-deprived pups.

B. Effects of deprivation in 12-day-old pups.

C. Deprivation effects in 18-day-old pups.

Figure 13. Patterns of mouthing activity to 10% sucrose infusions in 12-day-old pups after 24 hrs deprivation. Control pups received oral infusions alone while Gastric Load pups received oral stimulation and gastric loads as described in Fig. 11. From Swithers-Mulvey & Hall, 1991b.

Figure 14.

A. Patterns of mouthing in 12-day-old rat pups to a series of oral infusions of a flavored saccharin solution delivered once every minute or once every 20 seconds. Note that each point represents the average number of seconds mouthing expressed as a percentage of the inter-stimulus interval (mean \pm SEM). From Swithers-Mulvey & Hall, 1991a.

B. Intake (mean \pm SEM) in 12-day-old pups of a 4 minute continuous infusion of a flavored saccharin solution 30 minutes after the end of a habituation experience in which the interstimulus interval was 1 minute or 20 seconds.

* $p < 0.05$ compared to respective group None

$p < 0.05$ compared to respective group Oral/Switch

Figure 15. Patterns of mouthing responses of intact (control) and 24 hr deprived

decerebrate 12-day-old rat pups to brief oral infusions of 10% sucrose delivered once every minute. Each point represents the average (\pm SEM) number of seconds mouthing per minute over blocks of three trials. From Swithers-Mulvey, Mishu & Hall, 1991.

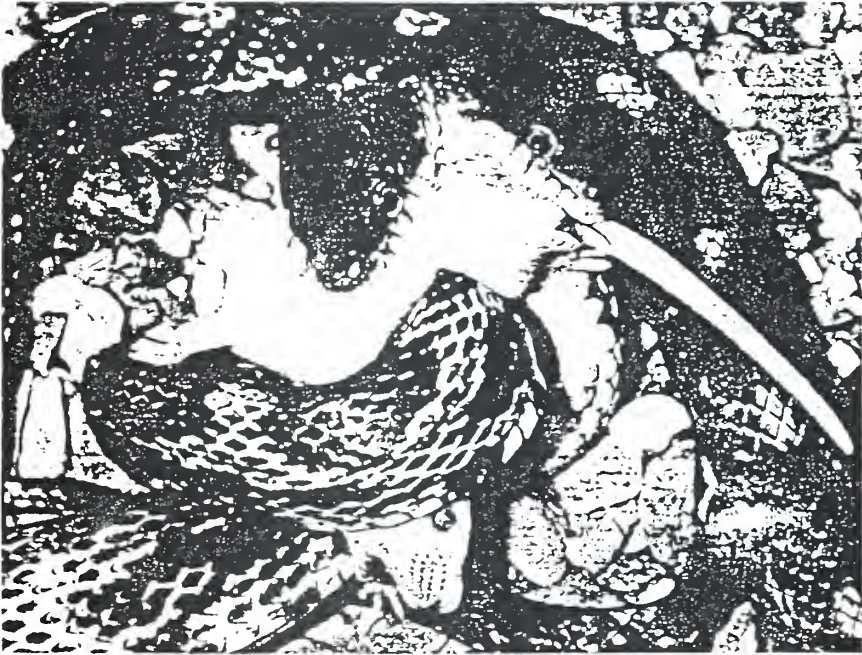
* $p < 0.05$ compared to control.

Figure 16. Mouthing activity in 24 hr deprived decerebrate pups to infusions of 10% sucrose. Each point represents the number of seconds mouthing per trial. Control decerebrates received oral infusions alone while Gastric Load decerebrates received oral infusions and gastric loads as described in Figure 11. Mouthing responses were lower in gastric load pups beginning with the fourth trial. From Swithers-Mulvey & Hall, 1991b.

Figure 17. Patterns of increased sham-fed intake of a liquid diet in 5 monkeys over 5 successive sham feeding experiences. Note a parallel rate of decrease in intake over time on each day of testing. From Gibbs & Falasco, 1978.

Figure 1

A.



B.

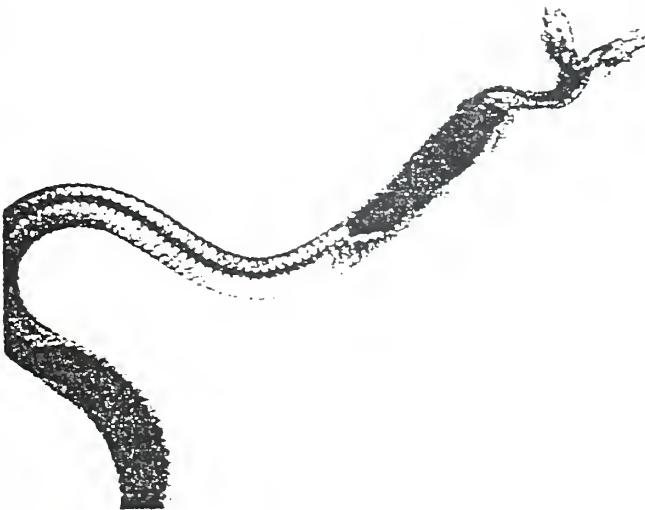


Figure 2

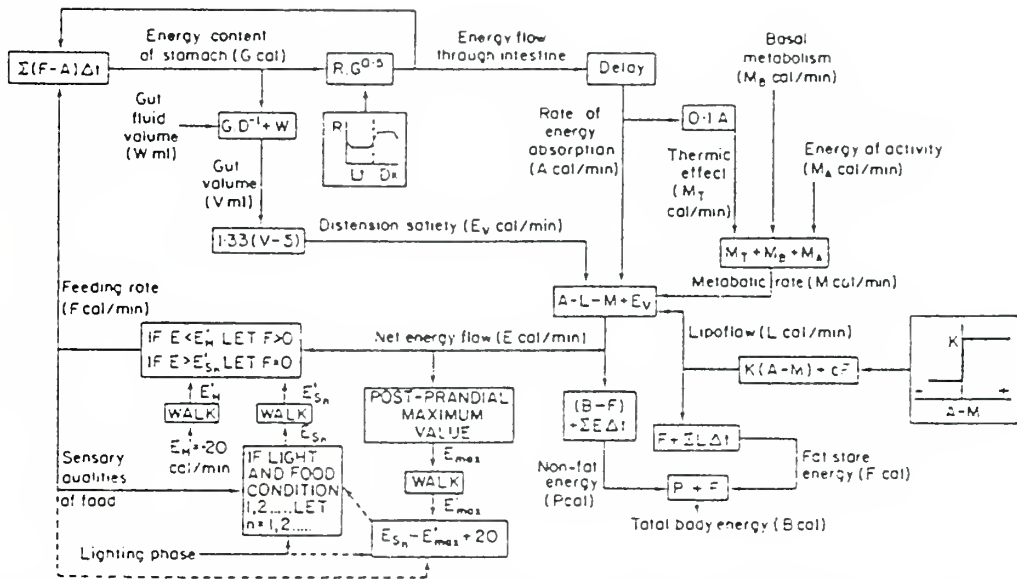


Fig. 13. Feeding model. Mark 3. The flow diagram defines the letter symbols used. "Walk" refers to the probabilized walk of hunger threshold, acquired satiety threshold and maximum energy flow recorded after a meal; these values shift around their mean values to represent the noise in the measurement of energy flow level by the hunger/satiety receptor system. Broken lines represent the retrieval of information from memory of the lighting conditions and sensory qualities of the food at the last feeding or of the acquired satiety value ($E_{s,n}$) which was established for the current lighting and foodstuff.

Figure 3

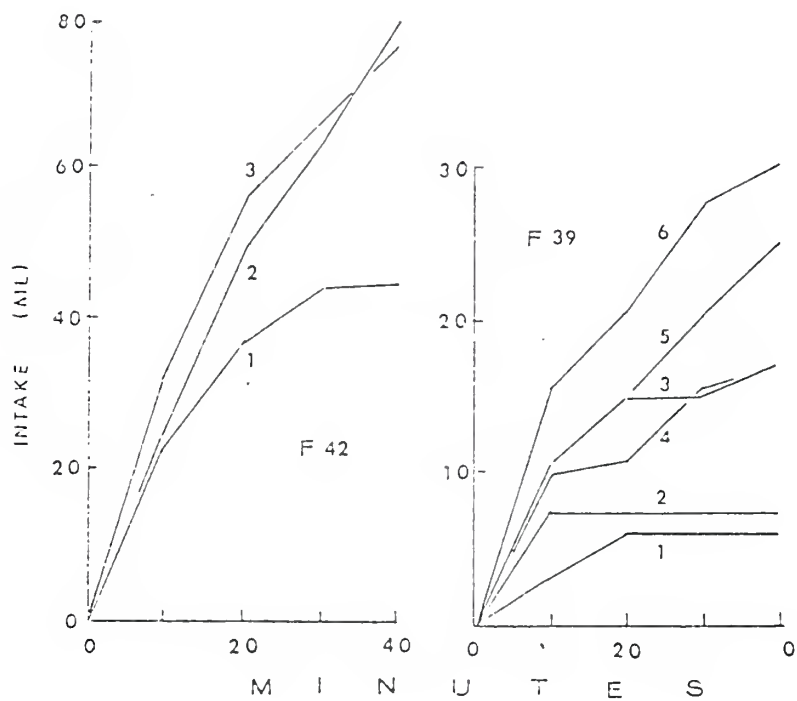


Figure 4



Figure 5

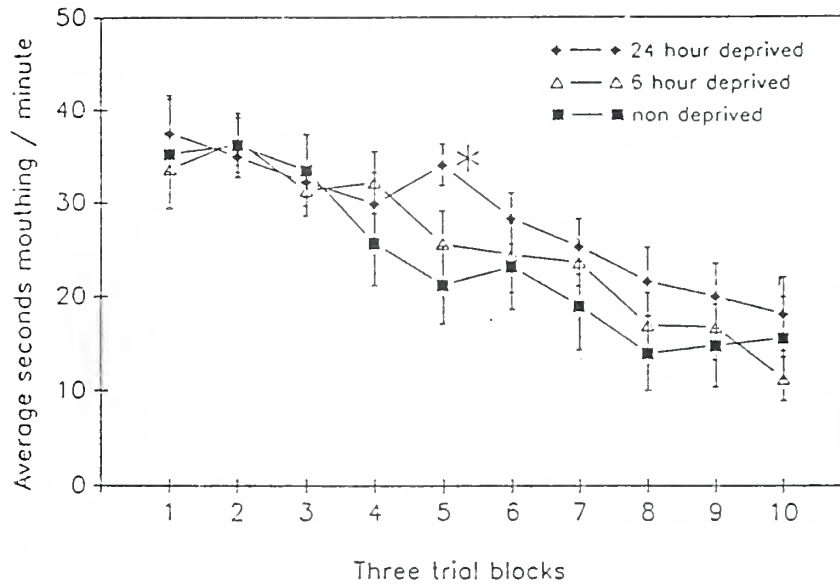


Figure 6

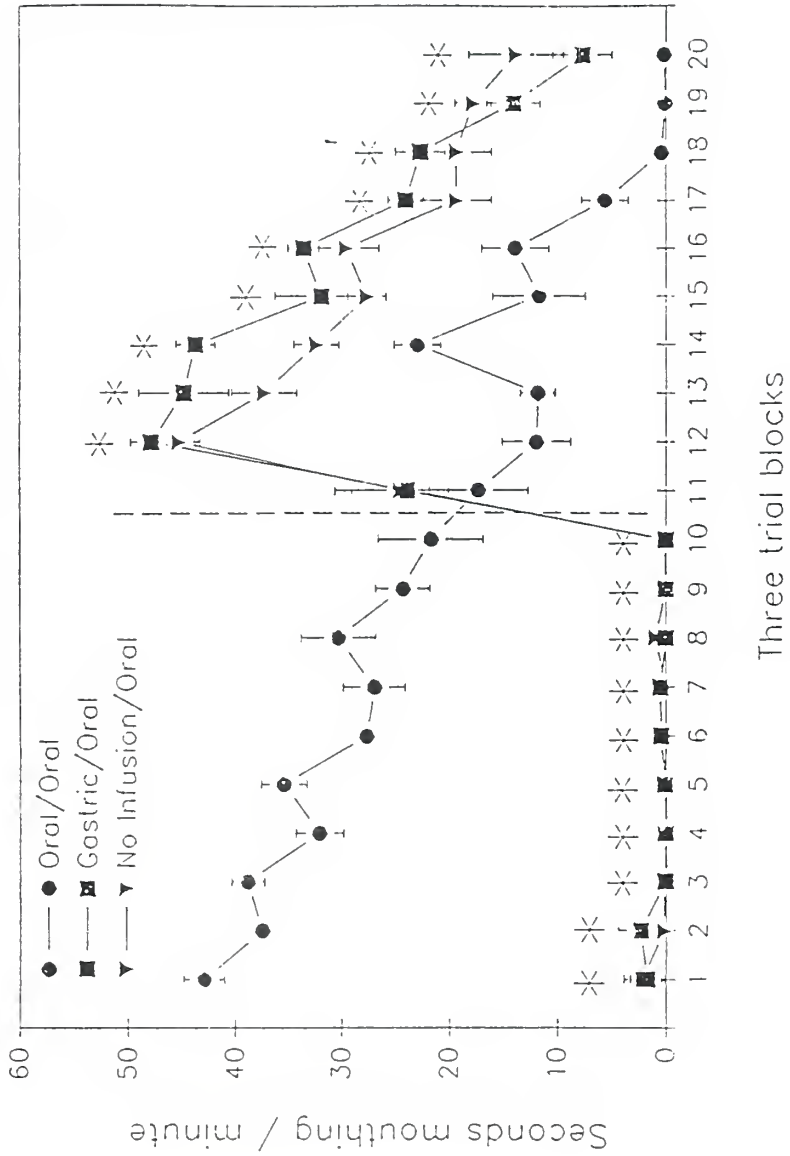


Figure 7

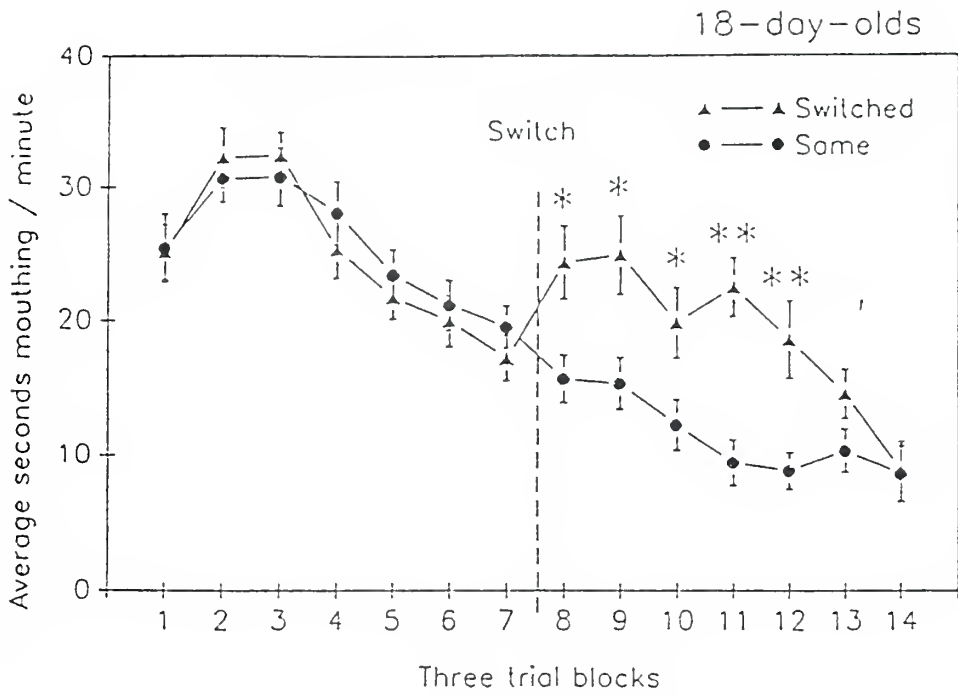


Figure 8

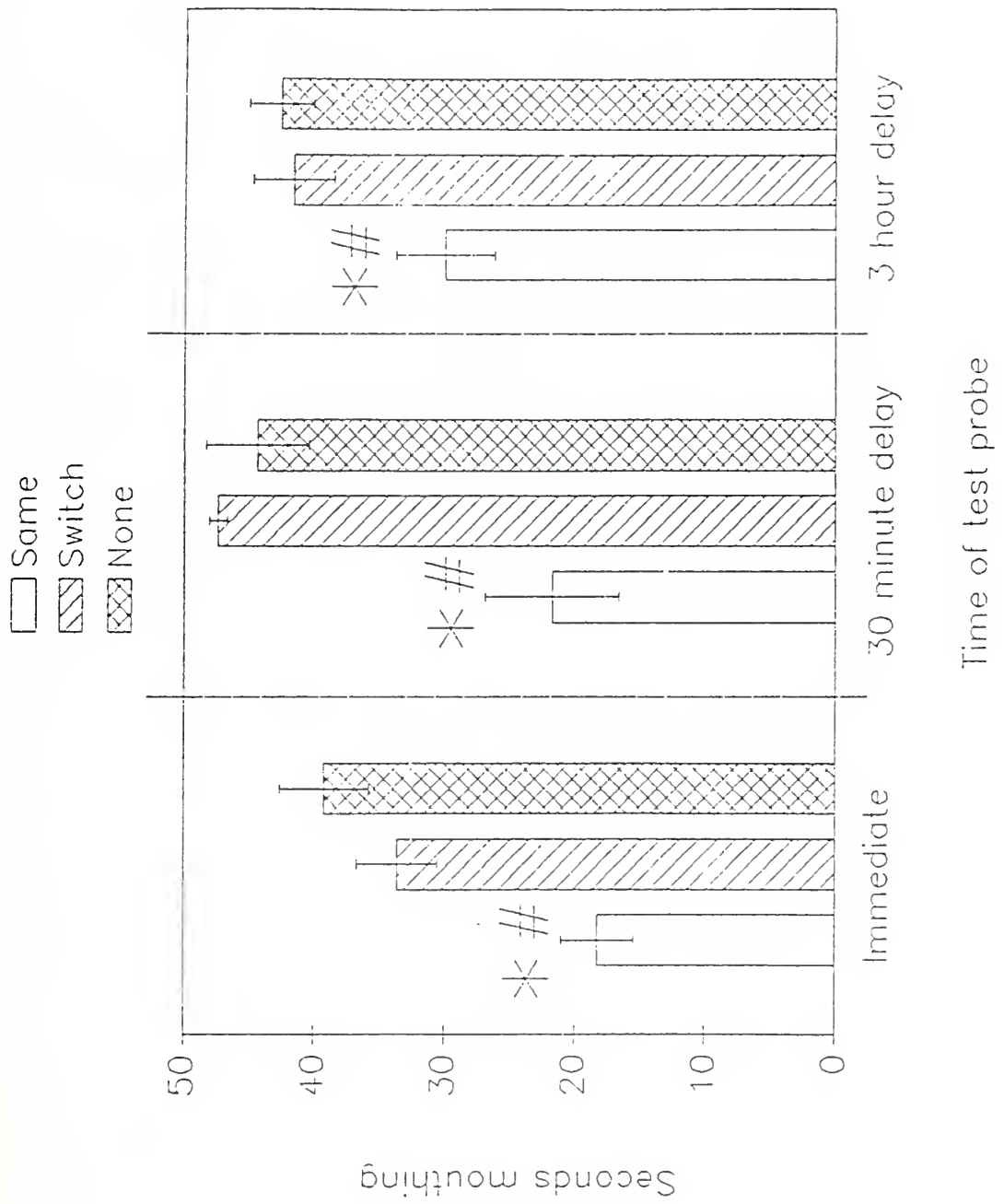


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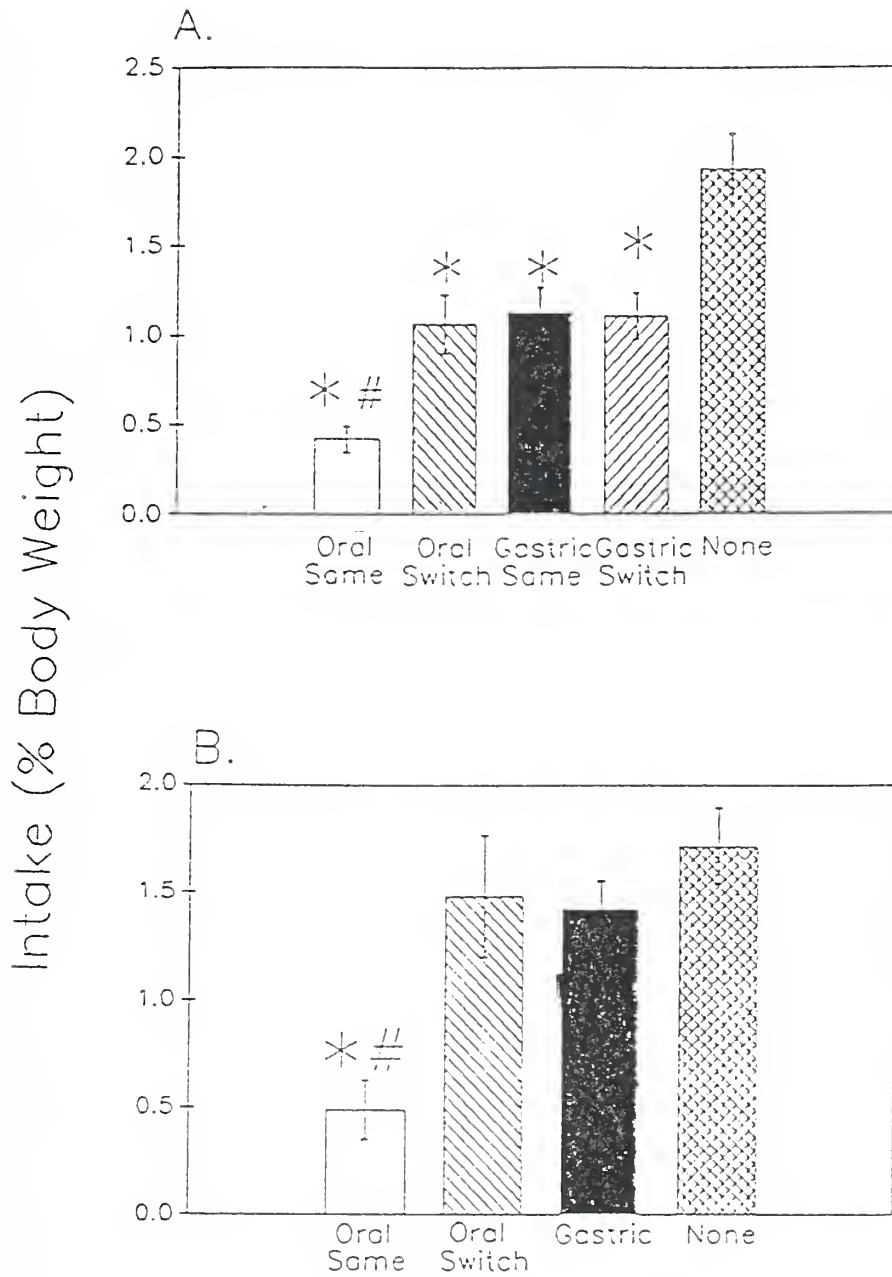


Figure 10

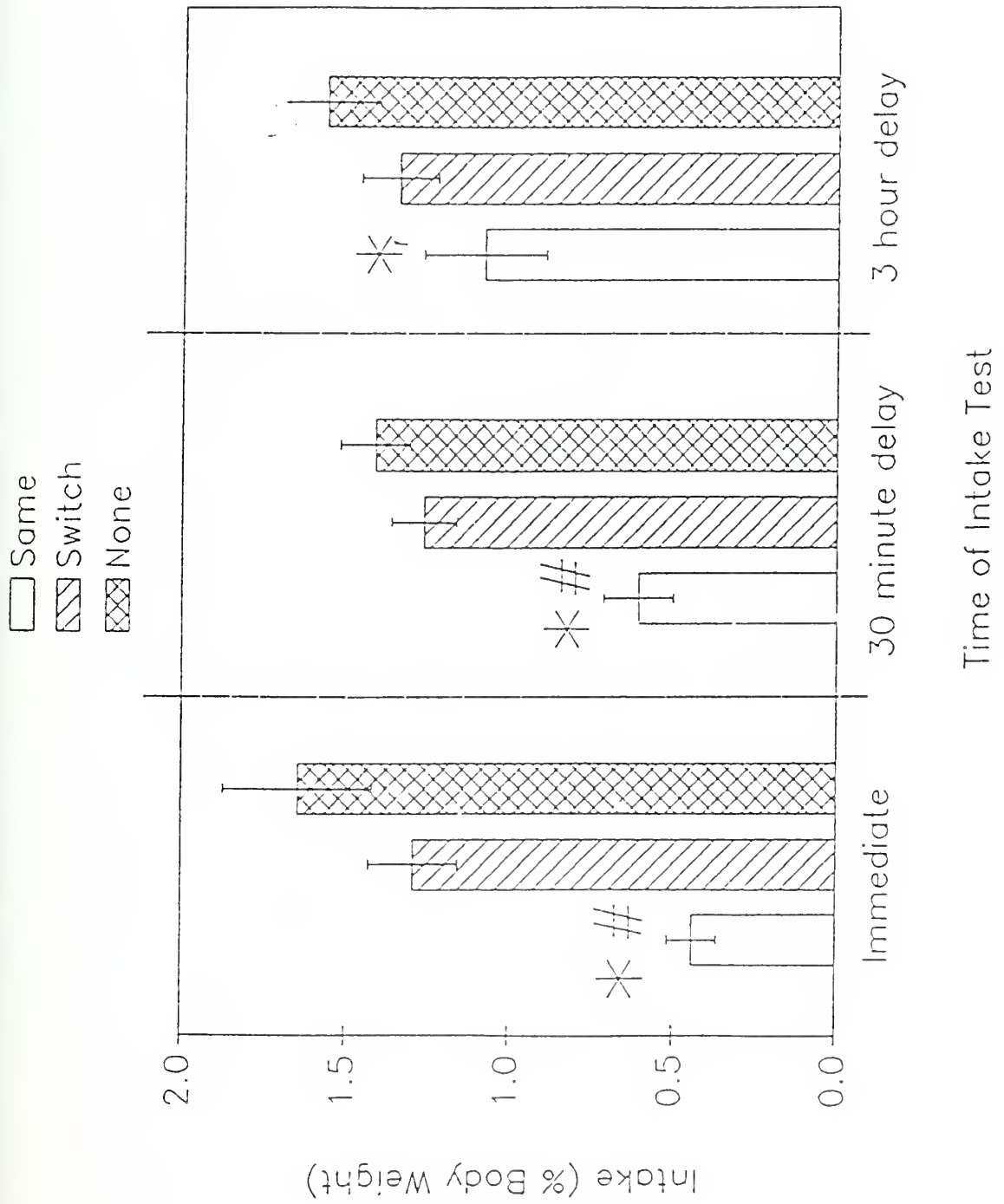


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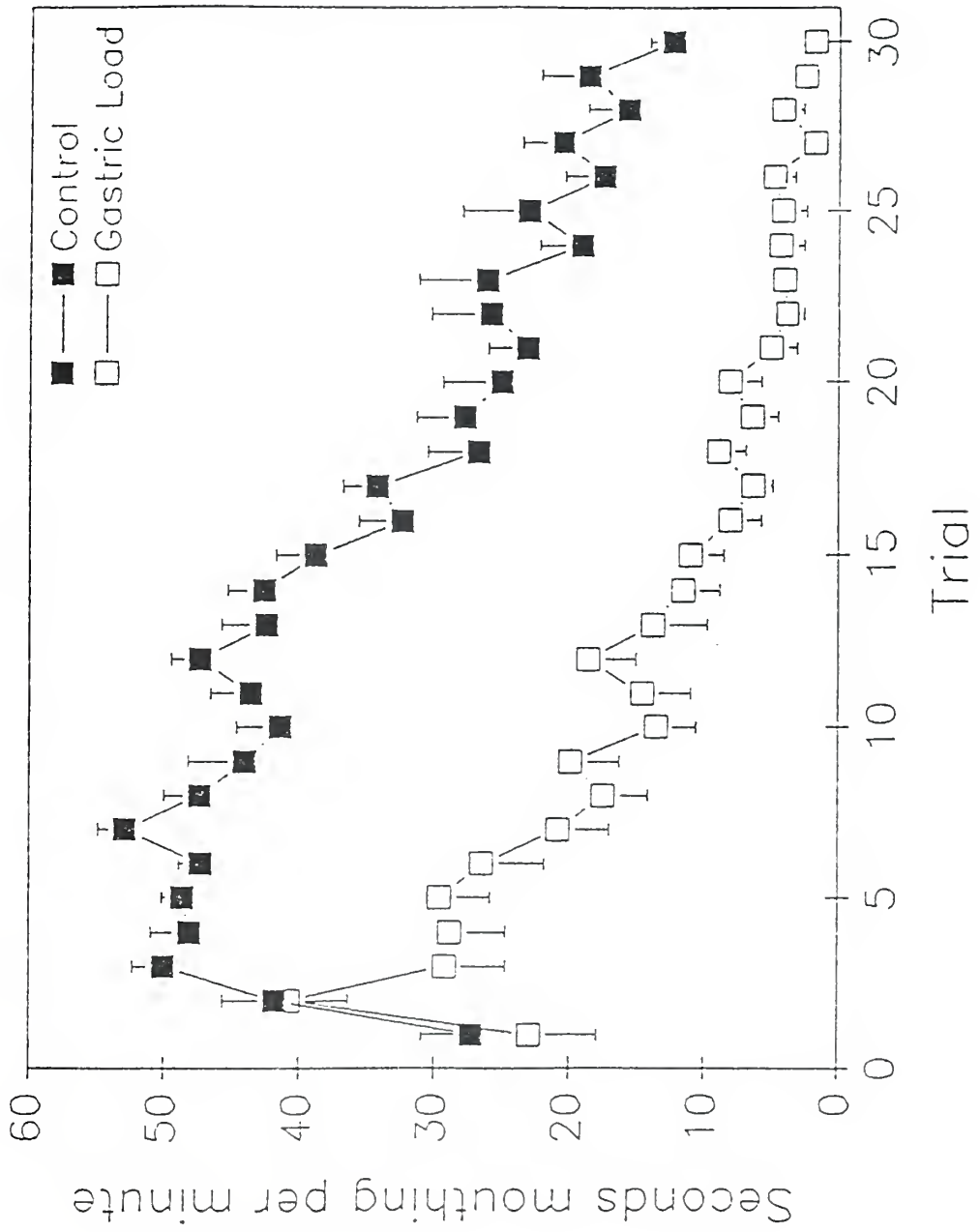


Figure 12

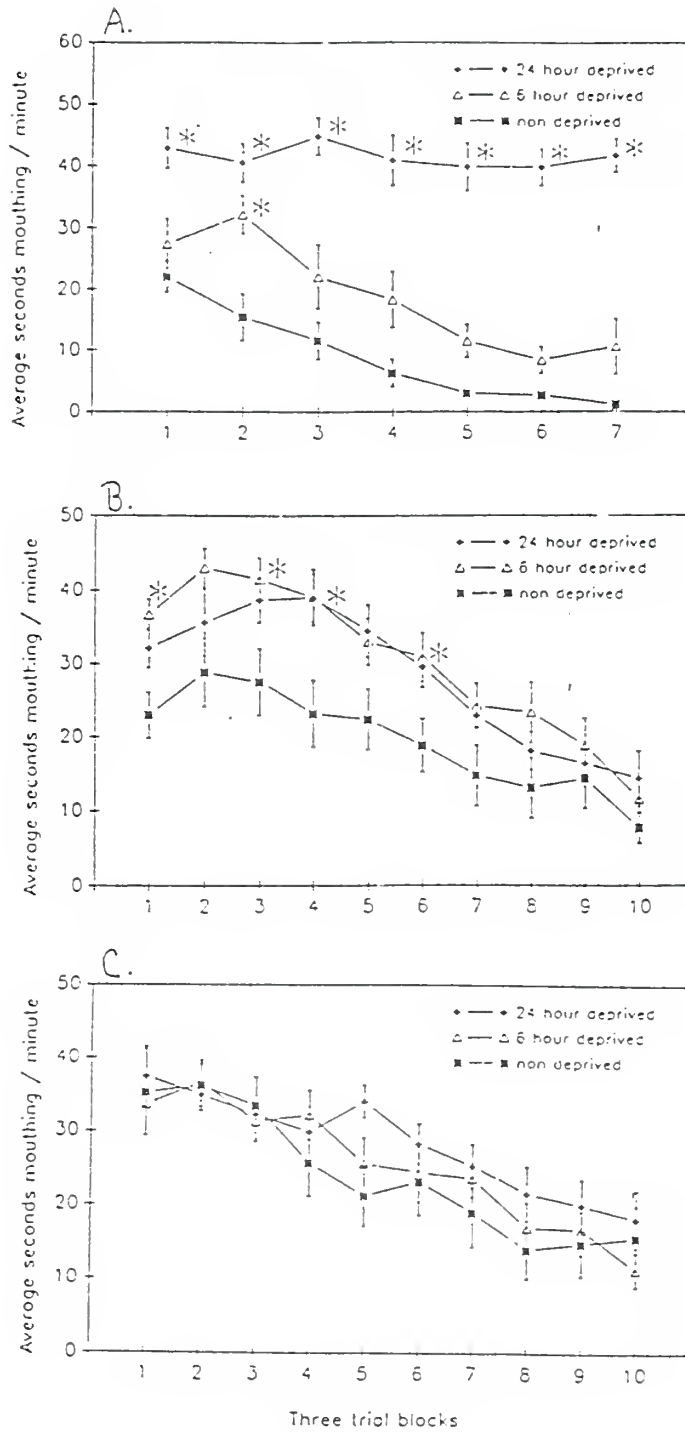


Figure 13

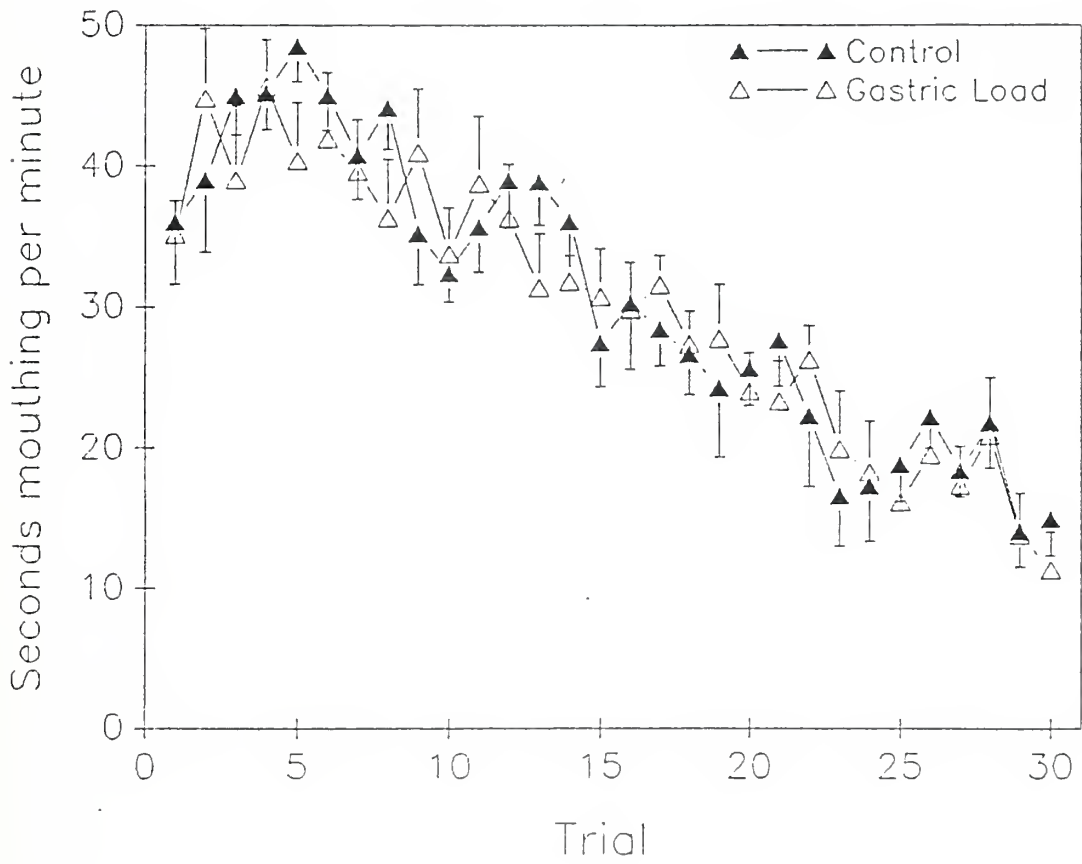


Figure 14

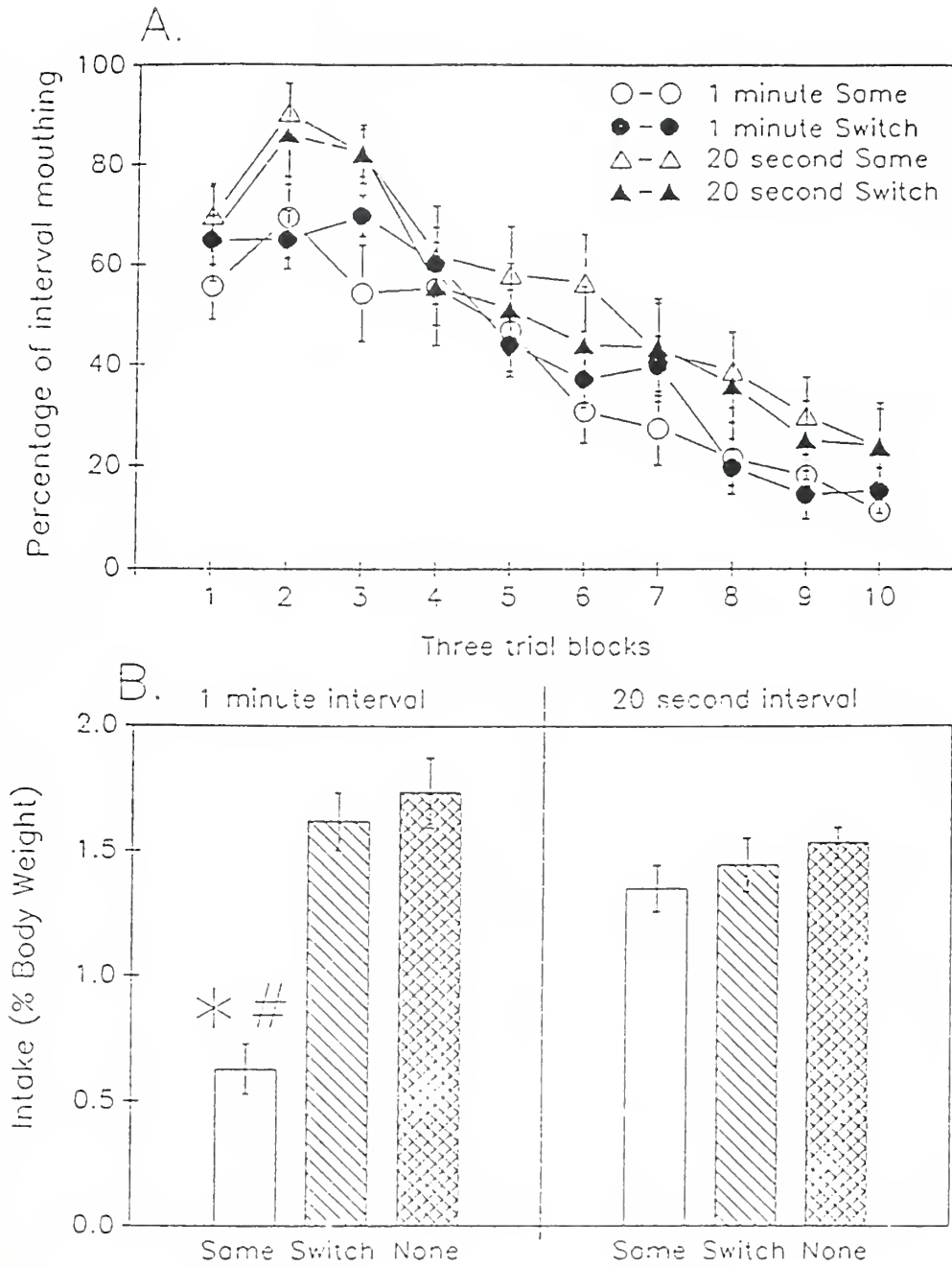


Figure 15

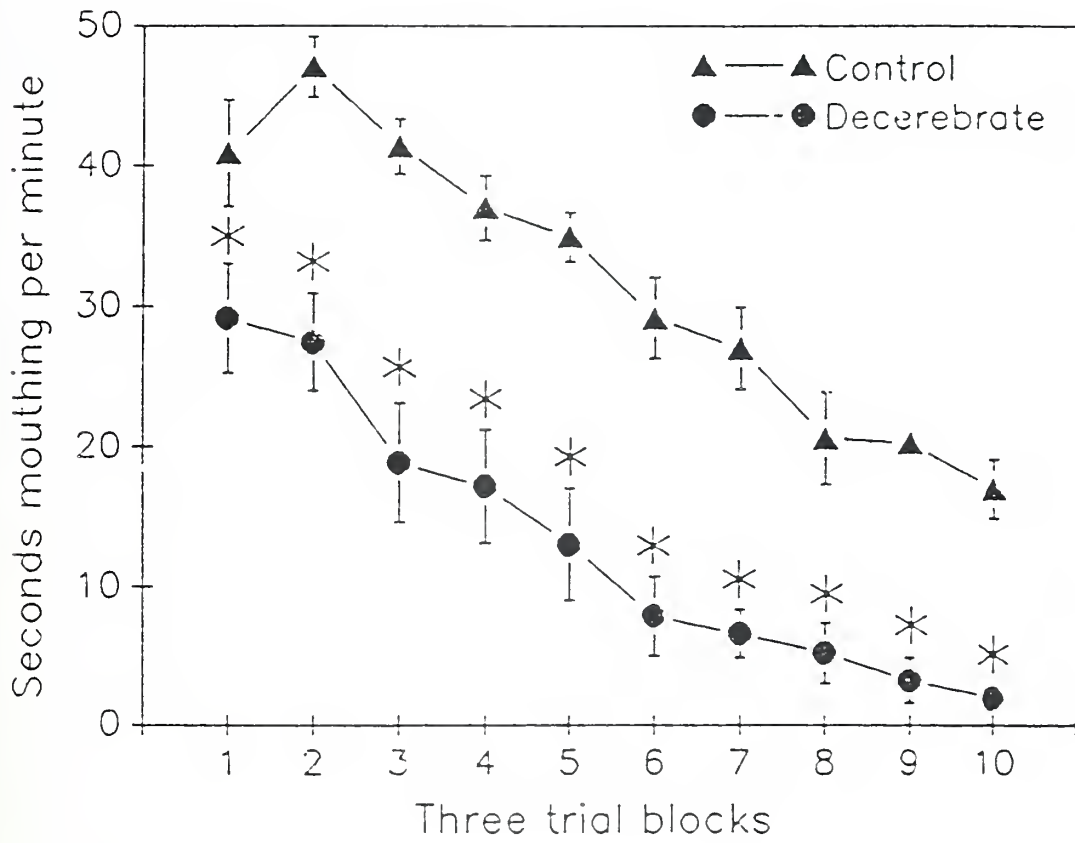


Figure 16

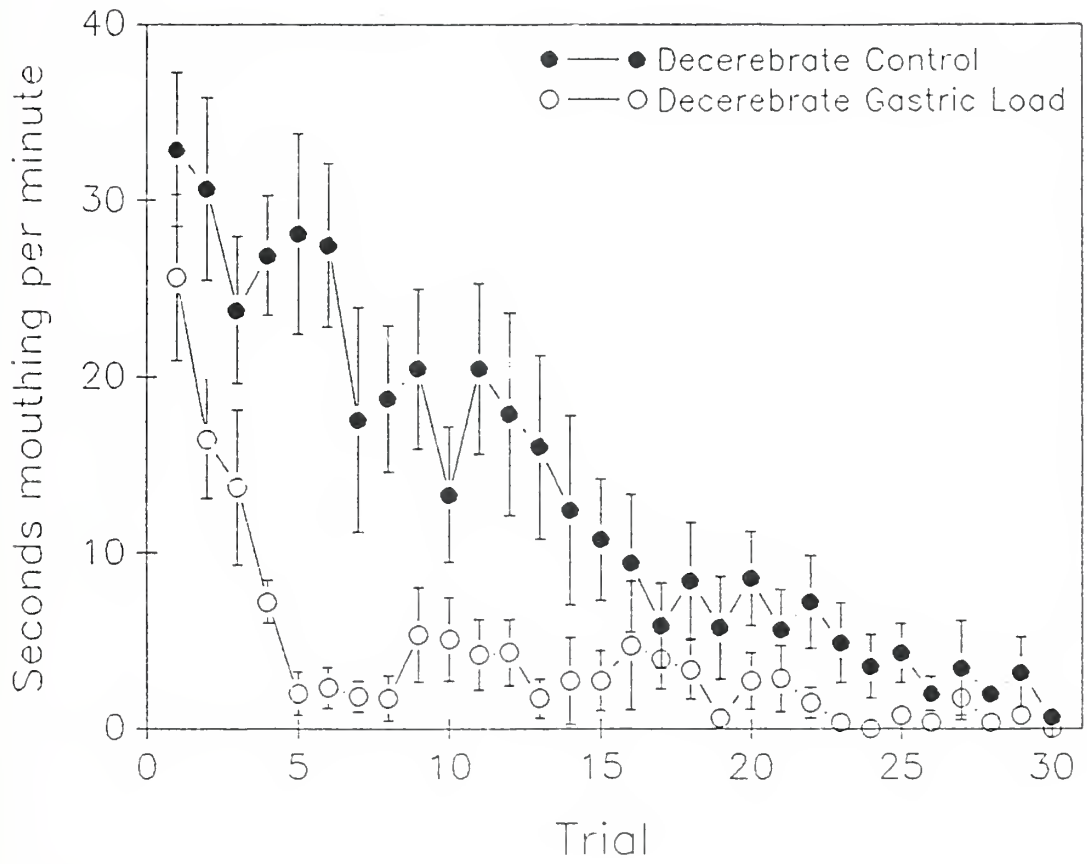
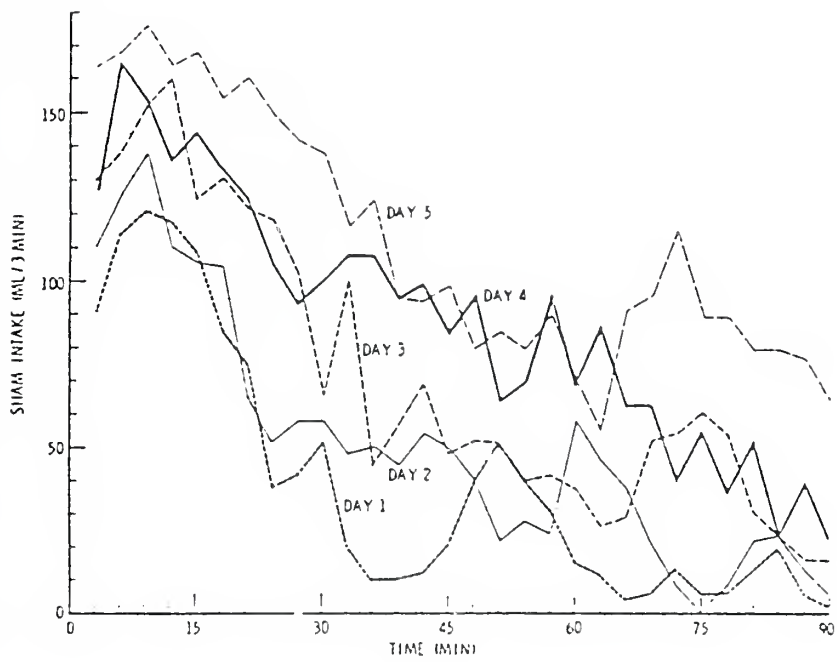


Figure 17



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Publications

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